

nature

October 9, 1975

Postgraduate education: MITs in Britain?

ON the same day that the Science Research Council (SRC) was launching its annual report (bearing, predictably, little good news for the practitioners of 'big science') it was also publishing a much less predictable document that makes some very radical suggestions. *Postgraduate Training* (SRC, free) should be read by every postgraduate, every academic and every industrialist in the business of hiring university-educated people.

A year and a half ago, the Commons Expenditure Committee looked at the postgraduate training scene in Britain across a spectrum from student funding to national relevance. It took advice from a strangely limited range of witnesses and came up with a dim view of the utility of higher degrees. The Department of Education and Science has been slow to reply to the criticism; in a sense this report can be seen as an attempt to remedy that deficiency. In no way, however, is it a stonewall defence of the *status quo*. The SRC working party, chaired by Sir Sam Edwards, has instead taken upon itself to inject ideas for change into the debate, and to propose major reorganisation at the post-graduate level.

The guiding principle behind the report is that postgraduate education should become less of a training ground for research workers and more of a broad continuation of the educational process. Several reasons are advanced. One is that the working party sees many scientific disciplines as having established a broad enough base to support technology "for a considerable time", and so there is now a greater need to train for careers for outside research. This point will cause alarm to some who will see it as the thin end of the wedge by which research, particularly in the physical sciences, is to be gently downgraded. Another reason for changing the emphasis of postgraduate training is to help persuade industry to take high qualifications more seriously. The working party has some hard words for the 'watching old Tom' school in which the sooner bright young students are out of university and learning the job by personal experience the better. A third reason, implicit in much that the working party proposes, is that there is a totally unsatisfactory and wasteful relationship between universities and professional bodies. It is farcical that in 1975 the working party has to suggest that the SRC enquire of these bodies whether certain university and polytechnic courses might be approved for professional training schemes. Fourth, even those who are ultimately to stay in research need education far beyond a first degree if they are not to become narrowly specialised. The provision of such education is at present patchy; many PhD students are able to avoid, or are unable to obtain, any formal training from the day they graduate.

The main recommendations are blunt.

- New, broader degrees to be available, both at master's and doctoral level, in which taught courses have an enhanced significance.
- A higher proportion of compulsory course work to be included in the first year of PhD courses.
- The option of proceeding to a PhD to be open only to those who have taken course work and in addition show aptitude for research.
- Consortia of universities, polytechnics and non-academic bodies to be formed to develop wider ranging courses.

If this looks much closer to the American pattern of postgraduate education, this is by design. The Edwards report is a curious echo of the Flowers report on university computer needs exactly 10 years ago. Then it was IBM which was the object of much admiration; now it is MIT. "We are anxious", says the report, "that the opportunities for graduate instruction offered by MIT should be matched by consortia of British universities, if not by individual universities."

And then finally, lest any academic applegarts be left un-upset, the working party recommend that postgraduates be paid differentially on the basis of the extent to which their work is of economic importance or in the national interest. Since the giving of grants is basically an SRC concern, such a measure could be rapidly implemented, although it is obvious that it could not be applied fairly except in some rather limited but clearly defined instances particularly favoured by SRC, such as joint SRC/SSRC studentships, total technology studentships and the Co-operative Awards in Science and Engineering (CASE) scheme.

The overall impression left by the report is certainly exciting and progressive, and it will speak directly to all those who know and approve of American higher education. But it all reads a little like a lecture, albeit a very good one, prepared by half-a-dozen lively minds gathered round a dinner table. Can the ideas be carried further than oratory? Is there any evidence, for instance, that the disappointing response to the CASE scheme would not be repeated on a much larger scale? Is there any indication that industry is prepared to accept more highly trained entrants? Do people say bad things about MIT's postgraduate education? Is a broad postgraduate training any use without broader undergraduate training than we have now?

That the working party is serious about its proposals, however, is in no doubt. "At a time when we are having to urge belt-tightening on everyone", says Sir Sam, "we wouldn't be proposing something as expensive as this were we not in deadly earnest". □





Nuclear fuel monitoring array at the Fast Flux Test Facility, Hanford, Washington

Rough passage in USA for first breeder reactor

The United States government is planning to spend more than \$10,000 million to develop a liquid metal fast breeder reactor. The programme is the highest priority energy research and development effort supported by public funds, but it has recently come under heavy criticism and it is beset by technical problems. In the second of a series of three articles, Colin Norman reports.

TO its supporters, who include President Ford and his predecessor, the liquid metal fast breeder reactor (LMFBR) could mean the difference between long term prosperity and economic disaster for the United States. But to its critics, who are legion, the reactor is an unacceptable health hazard and an unconscionable waste of taxpayers' money. The debate between those two camps, which has been reverberating for several years, has been getting more shrill lately, but about the only sure fact to emerge so far is that the federal government's costly effort to develop a commercially viable LMFBR is in trouble.

Two separate attempts to shut off funds for key elements in the LMFBR

programme were made in Congress this year. Although they were defeated, similar moves can be expected next year, when the programme's budget will be an even plumper target. Meanwhile, three Congressional committees have been studying the rationale for the programme, individual Congressmen have been taking well publicised pot shots at it, the General Accounting Office has recently issued a gloomy assessment of the effort's cost and timing, and a committee of the National Academy of Sciences is examining the project as part of a mammoth investigation of the entire nuclear power programme in the United States.

It is not difficult to pinpoint the reasons why the LMFBR programme has attracted so much controversy. For a start, it is the single most expensive energy research and development project supported by the federal government; it has also experienced huge cost overruns, has fallen badly behind schedule, and has encountered some nagging technical and safety problems.

But an equally important reason why the LMFBR has come under such concentrated hostile fire is that it is the centrepiece of the Administration's long term nuclear power programme. Without a commercial breeder reactor, the argument goes, light water reactors will run out of low-cost uranium within a few decades, but the LMFBR could prolong the use of nuclear fission as an energy source for several centuries. In other words, if the LMFBR is halted, the nuclear power programme itself will eventually be curtailed, which is reason enough for the breeder reactor programme to have become something of a rallying point for various environmentalist and anti-nuclear groups. Underlying the debate about the need for the LMFBR is a fundamental divergence of opinion on energy policy.

Critics of the programme argue, in short, that if the federal government takes some forceful steps to curb energy demand in the United States, the nuclear power programme could be slowed down. The result, they argue, would be that uranium resources would not be used up so quickly, the breeder reactor would not be needed so urgently, and thousands of millions of dollars which are now being spent on the LMFBR could be channelled into the development of more acceptable sources of power such as solar energy, geothermal energy and thermonuclear fusion.

Needless to say, such arguments are rejected by energy policy-makers in the federal government. They maintain that energy demand could not be cut back sharply without precipitating severe economic problems and that, in

any case, there is no guarantee that alternative sources of energy could be developed in time to preclude the need for the breeder. To cut off LMFBR development now, they argue, could put the United States in such dire straits early in the twenty-first century that the recent Arab oil embargo would seem, by comparison, like a mild inconvenience.

The situation was summed up last June by Dr Robert Seamans Jr, Administrator of the Energy Research and Development Administration (ERDA), the agency responsible for the LMFBR programme. Seamans said that "there is no presently available or prudent alternative" to pressing ahead with research and development on the LMFBR, in the hope that technical and safety problems can be solved in time for the breeder to provide "an essentially inexhaustible source of energy to satisfy a significant share of the nation's energy needs in the next century".

The LMFBR's promise of providing a cornucopia of energy rests on the fact that, by converting uranium wastes into plutonium during its normal operating cycle, it can 'breed' more nuclear fuel than it consumes.

Present-day reactors are driven by a nuclear chain reaction which involves the splitting apart of atoms of the uranium isotope ^{235}U . But unfortunately, only about 0.7% of naturally occurring uranium is in the form of that isotope, the remainder being the isotope ^{238}U which is incapable of sustaining a chain reaction by itself. Before it can be used as a nuclear fuel, natural uranium must therefore be put through an expensive enrichment process, which essentially consists of separating out some of the ^{238}U until the ^{235}U content reaches about 3-5% of the total. At present, ^{238}U removed during enrichment is simply stored as waste, but if it is put into the core of a breeder reactor it can be converted to ^{239}Pu by absorbing fast neutrons. Since ^{239}Pu is capable of sustaining a chain reaction, it can be used as a nuclear fuel.

By the time breeder reactors begin to operate in large numbers, it is argued, enough ^{238}U will have been stockpiled at uranium enrichment plants to provide fuel for the nuclear programme for several centuries. In other words, commercial introduction of LMFBRs would do away with environmentally destructive uranium mining, and would also eliminate the costly process of uranium enrichment. Moreover, because the ^{238}U will already be available, it will not fluctuate in price.

Because of such expectations, breeder reactors came under intensive investigation as long ago as the

1940s. In the mid-1960s, the Atomic Energy Commission chose the liquid metal fast breeder (so-called because it uses liquid sodium to transfer heat from the reactor core to steam generators) as the most promising design, and a similar choice was made in Britain, France, West Germany, Japan and the Soviet Union. Then, in 1971, former President Nixon made the LMFBR programme the highest priority energy research and development programme in the United States, a position it has enjoyed ever since.

The scope and timing of the programme have been revised several times in the past few years, but the latest plan being discussed in the ERDA is geared to achieving commercial introduction of the LMFBR in the early 1990s. The chief milestones on the path to that goal will be construction in 1978 of a test reactor called the Fast Flux Test Facility (FFTF), followed by a 350-MW prototype reactor which is now expected to be completed in 1983, and construction of a large commercial-scale plant capable of generating 1,000–2,000 MW of electricity in 1987. In addition, the ERDA is supporting extensive basic research and testing efforts, and two small experimental fast breeder reactors have already been operated, with mixed success.

Virtually every part of the programme has run into problems. Schedules have slipped badly, and the cost of the effort has skyrocketed. One of the most widely publicised facts about the programme, for example, is that estimates for the total cost of research and development on the LMFBR have increased from \$3,300 million in 1969 to about \$10,700 million now. Although ERDA officials insist that the vast gulf between those estimates can be explained in terms of inflation and by the fact that earlier estimates were not rigorously derived, the spiralling costs of the programme are generating embarrassing publicity.

As for individual parts of the effort, experience so far does not generate much confidence that the latest schedules and cost estimates will be met. The FFTF, for example, was originally expected to be completed by 1973 at a total cost of about \$87.5 million, but the latest completion date is 1978 and the cost is expected to reach at least \$600 million. Designed to test breeder reactor components in a high neutron flux, the FFTF will be a fully operational LMFBR, technically capable of generating 150 MW of electricity. It is now under construction in Washington State but will not actually be used for electricity production.

Similarly, the 350-MW demonstration LMFBR, which will be the next stage in the move towards com-

mercially operating breeders, has also suffered huge cost over-runs and delays. The original completion date of 1978 has slipped to 1983, and the estimated costs have increased from \$699 million to \$1,700 million. Construction of the reactor, in fact, has not even begun; site work is now expected to begin late next year at a location on the Clinch River near Oak Ridge, Tennessee. Recent attacks on the LMFBR programme have mostly been concentrated on the budget for this demonstration reactor, and more are likely next year.

The Clinch River demonstration plan was originally expected to be followed by a series of demonstration plants leading up to a commercial breeder reactor in about 1987, but earlier this year ERDA officials revamped and streamlined the programme considerably. The idea now is to build a large testing facility for LMFBR components in the early 1980s, and to follow the Clinch River plant with a single, commercial-sized reactor in 1987. Design of that reactor will begin next year.

As recently as last March, ERDA officials told Congress that if the LMFBR programme can stay on its present schedule, more than 100 breeder reactors could be operating commercially in the United States by the year 2000. But even that estimate has since been revised. In a long range plan for energy research and development which was published by the ERDA on June 30, it was suggested that only 30–40 breeders could be anticipated by the end of the century. The breeder's impact, that report indicated, will not be felt until early in the twenty-first century.

With all those cost and scheduling problems to shoot at, the LMFBR's critics have never found themselves short of ammunition, and their assaults are expected to intensify in the next few years. The chief source of criticism at the national level is the Natural Resources Defense Council (NRDC), a public interest group whose chief activists against the breeder are Dr Tom Cochran, a nuclear physicist, Dr Arthur Tamplin, a former health physicist with the Atomic Energy Commission, and Gus Speth, a lawyer. In the past few years, the NRDC has generated reams of critical analysis of the federal government's justification for the programme, and it has received some backing on a few of its charges from the Environmental Protection Agency (EPA).

Aside from the cost of the LMFBR programme, the chief disputes will revolve around the following themes. **Uranium resources.** The ERDA has calculated that there is only enough low-cost uranium available in the

United States to provide about 30 years' worth of fuel for each of the light water reactors which will be built before the mid-1990s. In other words, domestic uranium reserves will be totally committed to those nuclear reactors which are brought on line in the next 20 years, and reactors built after about 1995 will begin running out of fuel before their useful life is finished. The ERDA therefore argues that the LMFBR's nuclear breeding capabilities will be needed to prevent the nuclear power industry from grinding to a halt.

The NRDC has challenged those assumptions on two grounds, however. It has argued that the ERDA has adopted unrealistically high estimates for the growth of energy demand, and that it has adopted unrealistically low estimates for the size of uranium reserves, both of which make commercial introduction of the breeder seem more urgent than it really is. Cochran argued before a Congressional committee last June that the commercial component of the breeder programme could be delayed for a decade without any penalty, and that the Clinch River demonstration plant should therefore be cancelled. He suggested that the delay would allow time for more accurate assessments of energy demand and uranium supply, and that the money saved could be better spent on developing solar, geothermal and thermonuclear fusion energy.

Those views have received partial support from the EPA, which suggested in a report last June that commercial introduction of the breeder could be delayed for up to 12 years without incurring economic costs. The ERDA argued, however, that work on the Clinch River demonstration plant should proceed as rapidly as possible in order to help solve some of the more pressing technical problems.

Officials from other government agencies have argued that since there is such utter confusion about long term energy forecasts, it would be foolish to slow down development of the breeder at this stage. The present policy is, therefore, to forge ahead with research and development on the LMFBR and to leave until a later date a decision about whether, or when, the reactor should be introduced commercially.

Plutonium. A large commercial LMFBR programme would inevitably entail the processing, transportation and use of huge quantities of plutonium. And that is a prospect which is worrying many people.

Tamplin and Cochran suggested in a petition to the former Atomic Energy Commission last year that if tiny inhaled particles of plutonium lodge in

the lung, they will give a massive dose of radiation to a tiny area of surrounding lung tissue. They argued that since present standards governing exposure to plutonium are based on the assumption that radiation from inhaled plutonium can be averaged over the entire lung, the permitted dose should be reduced by a factor of at least 100,000.

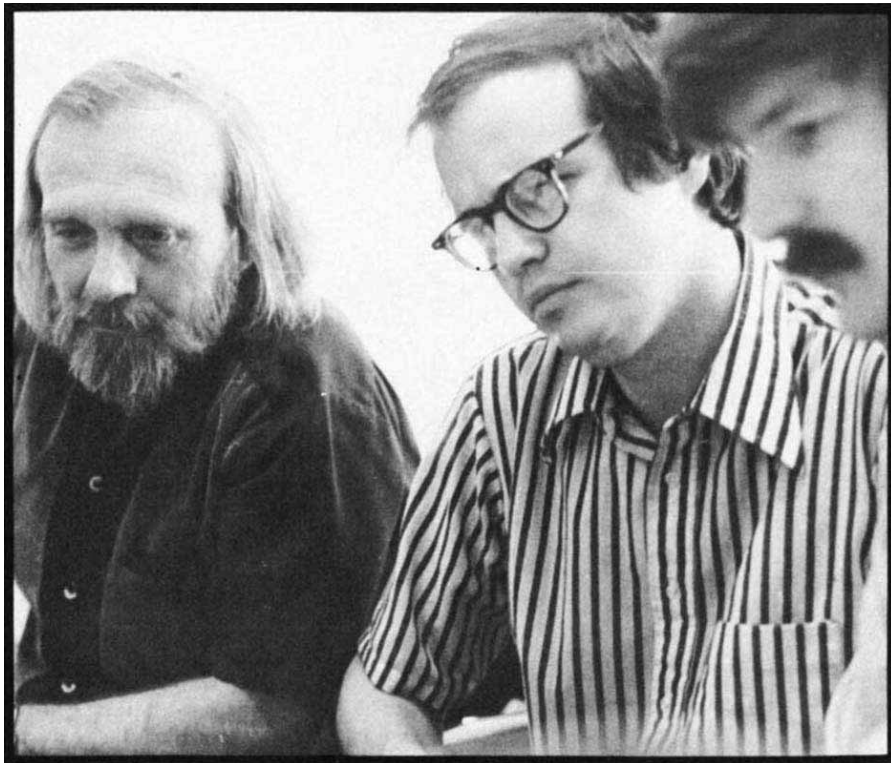
Although Tamplin and Cochran's argument has been hotly contested by scientists from the former Atomic Energy Commission and, independently, by Britain's Medical Research Council, their theory has continued to give rise to considerable discussion.

Public concern over plutonium toxicity has recently given way to even deeper concern about the possibility that plutonium could be stolen and fashioned into a crude atomic weapon. It is generally accepted that present procedures to guard the theft of potential bomb ingredients would be unsuited to a large scale plutonium industry. For that reason, the Nuclear Regulatory Commission (NRC), which is responsible for regulating the nuclear industry in the United States, recently decided that it could not yet allow plutonium to be recycled as a fuel for light water reactors. That decision will probably have to wait until 1978, the NRC says, when a number of studies of safeguard measures will have been completed.

Fears about plutonium toxicity and safeguards will obviously have to be resolved before a commercial LMFBR programme is allowed to make its debut.

Reactor safety. Although ERDA officials insist that LMFBRs would probably be even safer than light water reactors (chiefly because the liquid sodium coolant has excellent heat transfer properties and because there is a large temperature difference between the melting and boiling points of sodium), nagging doubts about the safety of breeders nevertheless remain.

And those doubts have not been assuaged by operating experience with one of the two experimental fast breeder reactors which have been constructed in the United States. The Enrico Fermi Atomic Power Plant, a 34-MW experimental reactor built in the late 1950s at Lagoona Beach, Michigan, suffered a partial core meltdown in 1966 because of a blockage in the coolant system. The accident, which resulted in radioactivity leaking from the primary containment building, was long considered the most serious mishap in the history of the nuclear power programme. Although ERDA officials rightly insist that such an accident would be extremely unlikely to occur again, the incident has nevertheless been something of a public embarrass-



Tamplin, Speth and Cochran: reams of criticism

ment to the fast breeder programme.

Officials of ERDA maintain that, in the extremely unlikely event that the nuclear core of an LMFBR should melt, the primary containment vessel which surrounds the core would prevent radioactive material from leaking into the environment; a small-scale test on a model of the FFTF has supported such an expectation. Nevertheless, a statement published by the former Atomic Energy Commission early this year acknowledged that "it must be emphasised that technical uncertainties remain to be resolved and work remains to be done before this expectation for systems other than those for which tests have been performed can be thoroughly assessed". And ERDA Administrator Seamans notes that safety questions "must be resolved satisfactorily before any decision may be made to place LMFBRs into commercial use".

Although debate over such issues has been going on in the United States for such time, Congress has only recently begun to examine the LMFBR programme in any detail. Until this year, for example, Congress has been happy to approve hundreds of millions of dollars for the programme without subjecting the matter to any discussion outside the tender confines of the Joint Committee on Atomic Energy.

But the massive cost escalation in the programme has changed all that. Farlier this year, amendments were offered to ERDA's budget authorisation bill which would have halted most of the spending on the Clinch River

demonstration plant for at least 18 months, to allow time for an independent study of the need for the LMFBR. The move failed in the House by 227 votes to 136 and in the Senate by 66 votes to 30, but critics of the programme are not disheartened. Pointing out that they have already received support from a third of the Congress after only a year's effort, they have promised to try again next year.

There is, however, only a small possibility that the LMFBR programme will be halted outright by Congressional action. For one thing, Congress would be reluctant to close down a programme which has such a large potential for meeting energy needs. And for another, the fact that several other countries are vigorously developing LMFBRs will provide a strong incentive for Congress to continue with efforts in the United States. There is also the fact that hundreds of millions of dollars have already been devoted to LMFBR development, an investment which would be difficult simply to write off.

But there is one other factor which is worth bearing in mind. If the thermonuclear fusion programme continues to make sound progress, as it has in the past few years, it will soon require massive outlays on new machines and it would then be competing directly with the LMFBR programme for funds from the ERDA's budget. The LMFBR programme has so far received priority because it is closer to commercialisation, but a significant breakthrough in the fusion programme could, conceivably, change all that. □

international news

BRAZIL, which gained world attention earlier this year by signing a unique nuclear technology pact with West Germany, has outlined an ambitious nuclear energy programme that will take it up to the year 2000. The president of a government-run electricity company called Furnas Centrais Elétricas SA, Luiz Claudio de Almeida Magalhaes, announced that by the end of the century, Brazil will have 63 nuclear reactors, with a total generating capacity of 81 million kW.

Magalhaes unveiled this plan in a lecture last month at the Superior War College, a strategy and policy 'think tank' for key civilians and military men which is regarded as highly important by the military-controlled government that runs Latin America's biggest nation. This speech came barely two months after the signing in Bonn of the Brazilian-German agreement, which provides for the transfer to Brazil of the know-how for enriching uranium and building nuclear power plants, along with the actual construction of eight nuclear reactors in Brazil.

Magalhaes explained that although Brazil has immense hydroelectric potential, it won't be enough to take care of the nation's voracious appetite for electricity after 1990. The country already possesses some of the biggest hydroelectric dams in the world, and it has just started building the biggest of them all, a 12-GW giant called Itaipu, across the Parana River, which should go on stream in the mid-1980s. Nonetheless, Brazil must think even further ahead.

A major problem for the development of Brazil is that its booming major cities are located either in the south-eastern part of the country or along the Atlantic coast, whereas its principal untapped rivers, in terms of hydroelectric power, are in the Amazon jungle, 2,000 miles away. And Brazilian urban areas are growing at a mind-boggling rate.

Sao Paulo, the country's industrial capital, has a city-proper population of 7 million and should overtake New York as the Western Hemisphere's largest city within a few years. The Sao Paulo metropolitan area at present has over 9 million people, and even conservative forecasts say this will increase to around 20 million by the end of the century. Rio de Janeiro has a population of nearly 5 million people, not counting the suburbs. Other

Brazil plans 63 nuclear reactors this century

from Bruce Handler, Rio de Janeiro

Brazilian cities such as Belo Horizonte, Recife, Porto Alegre, Salvador and Fortaleza—which most outsiders have never heard of—have already reached the 1 million mark.

Since Brazil produces only around one-fifth of the crude oil it needs every year, there is only one solution for its future energy requirements; nuclear power. Brazil's first nuclear generating station, a 627 MW installation at Angra dos Reis, 130 miles from Rio, is to begin operating in early 1978. This plant was built by Westinghouse Electric of the United States, but because of restrictions imposed by the US government, Brazil did not acquire any nuclear technology in connection with the project and it had to send uranium to the United States to be enriched. Because of Brazil's undeniable need for nuclear energy in the future, Brazilian government officials saw the nuclear security limitations dictated by Washington as extremely prejudicial and encumbering and so turned to Germany for nuclear expertise.

Two German-designed reactors of 1.3 GW each are to be built at Angra dos Reis, practically next door to the American plant, and they should come into operation in the mid-1980s. Six more German reactors, all in the 1.3-GW range, are to go on stream by 1990, serving Sao Paulo, Rio, Salvador and Recife.

Magalhaes did not say who would design and build the 54 additional reactors planned for the year 2000. Theoretically, by 1990 Brazil will have learned all the tricks of the trade from the Germans and could design the remaining reactors itself, contracting out specialised work when necessary. The Brazilian energy official said the 54 reactors will all be near big cities.

Magalhaes told his audience at the Superior War College that Brazil would be "extremely vulnerable" if it depended too heavily on hydroelectric power, although he did not elaborate on this. His main objective was to convince possible sceptics that Brazil did the right thing by making the big nuclear power push now. Magalhaes noted that Brazil's "useable" hydroelectric potential will run out by the end of the century. On paper, Brazilian rivers could provide 120 GW of power (the capacity installed at present is around 15 GW). But Brazilian officials, who have studied hydroelectrical projects in the Soviet Union, which has similar problems of distance, are less than enthusiastic about the high cost and inefficiency of long power lines.

Magalhaes was also bluntly unenthusiastic about Brazil's chances of significantly increasing oil production. The state oil company, Petrobras, which has a monopoly on drilling and refining, has been spending about \$275 million a year on prospecting, but production has remained about the same for the past several years. There are encouraging off-shore oil finds but, in spite of fantastic predictions of self sufficiency by certain government officials, it still seems too early to tell how good these new discoveries are. Meanwhile Brazil continues to spend more than US\$3,000 million a year on expensive crude oil from the Middle East. There have been suggestions that Petrobras should let big foreign oil companies look for oil in Brazil under service contracts but these have been rejected, mostly for nationalistic reasons.

At almost the same time that Magalhaes was explaining why Brazil has no choice other than nuclear energy as its prime source of energy in the future, the Mines and Energy Minister, Shigeaki Ueki, announced two new uranium discoveries. A big question mark in the Brazilian-German deal is whether Brazil actually has enough uranium to be able to carry out its ambitious plans for nuclear power. The minister said that two uranium deposits had been located in the central state of Goias and that rough preliminary estimates indicated a total of 1,500 tons of uranium ore. If this estimate holds up under further investigation, it will mean a 14% increase in Brazil's known uranium reserves. □

THE Science Research Council (SRC), hit for the second consecutive year by a reduction in its spending power, is to review its allocation of funds in an effort to maintain a balanced research programme in Britain. Several measures have been suggested, among them the provision of a larger proportion of available funds to university research, but the most interesting innovation in the long term is the introduction of industrially based research programmes designed to attract investments from commercial interests.

Speaking last week following the publication of the council's Tenth Annual Report, the Chairman of the SRC, Sir Sam Edwards, ranged freely over the council's record during the past year and its strategy for the year ahead, and made particular play of the newly approved polymer research programme established to encourage cooperation between academic and industrial interests. Spread over a period of five years, the project will operate on a budget of £2.5 million, which will include contributions from the British Plastics Federation and the British Rubber Manufacturers' Association, and will be responsible to a directorate that will "initiate and oversee a closely coordinated research programme" involving both the universities and industry.

To judge from Sir Sam's enthusiasm, the council sees the adoption of the new programme as a significant development in the running of research and plainly hopes that industrial interests will eventually be encouraged to provide support for similar programmes from which they can ultimately expect to benefit.

Certainly, the council's breakthrough in that area of research funding has come at an apposite moment. Because of inflation, the council's budget for 1974-75, just over £85.5 million, was 2% lower in real terms than in the previous year; a similar reduction is forecast for 1975-76. The effects of inflation are, moreover, aggravated by the council's commitment to the European organisations CERN and the European Space Agency (ESA), which is guaranteed in Swiss currency and which has taken up an increasing share of the budget as the pound sterling has weakened. To make the best of the remainder of the budget, which is divided between bread-and-butter support for research and facilities in the universities, and the financing of major projects which require a high expenditure in a single area of research, the council is to implement cuts in the latter sector.

Already the council has completely abandoned two of the fifteen major

projects listed in last year's report—the European X-ray satellite and the Mark VA telescope—in spite of their admitted scientific importance. And of the seven listed in the present report (which include the Northern Hemisphere Observatory and the Electron-positron Intersecting Complex—EPIC) Sir Sam declared that he could see at least two of them "going overboard" during the coming year. Questioned on which they might be, he chose not to show his hand, adding only that it

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would cost about £74 million to adopt all seven whereas the sum available would be nearer last year's figure of £25 million. It seems reasonable to speculate, however, that with the rising cost of space research the NASA satellite project will never see the light of day.

The council's affirmation of support for university research is highlighted by the timely approval of the laser laboratory which is to be established at the Rutherford Laboratory in Chilton, Oxfordshire, at a cost of £5.7 million. It will provide a central research facility for the universities and polytechnics. The original involvement of the UK Atomic Energy Authority in the project, which was from the outset somewhat uncertain, has now been withdrawn altogether.

As far as research training is concerned, the number of postgraduates studentships will be reduced by 300 to about 3,600 in the coming year. This cut comes in spite of a 25% increase in the number of applications and means that only 14% of science and engineering graduates will be catered for. Nonetheless, the council has not been entirely negative on that front, having proposed a facelift for research training (see page 433).

More generally, the report announces the council's intention to "sharpen" the criteria used in the selection of particular fields for research funding, and though Sir Sam was characteristically phlegmatic on what precisely that means, the report itself indicates that it is the programmes considered of national importance that will receive the most favourable consideration.

● The decision to press on with the German ring accelerator PETRA in Hamburg sounds like the death-knell for its British competitor EPIC. Although the commitment of 14 million Deutschmarks is only for buildings to house the machine, approval for the machine itself cannot be far behind. Dr Godfrey Stafford, director of the

Rutherford Laboratory, at which EPIC would have been housed, was not only disappointed at the German decision, but feels it has some unfortunate implications for the future of high-energy research. The European dimension to such research, represented by CERN, and which he believes EPIC, with international funding support, would have maintained, is in danger of reverting to national rivalry. Recent unilateral initiatives by the French (with GANIL) and now the Germans cannot but put more global programmes under serious pressure.

● The United Kingdom Atomic Energy Authority (UKAEA) also presented its annual report last week, and the general tone of the peroration of its Chairman, Sir John Hill, was one of relief at the end of several years of bickering over the way British nuclear policy should proceed. During the past year the government decided to go for a steam generating heavy water reactor, while maintaining the national effort on the fast reactor; during the coming year the first two advanced gas-cooled reactor (AGR) stations at Hinkley Point and Hunterston should come on line, and the prototype fast reactor, which has been delayed by various teething troubles, should come into full operation. After 10 years of vast overspending, delays running into years, and an industrial structure which was, to say the least, unhelpful to the production of any kind of energy, the authority's chairman was wiping his brow and brightening at the prospect of a New Era in nuclear engineering. But if the progress of the £45 million Prototype Fast Reactor at Dounreay is anything to go by, the UKAEA's optimism could turn out to be the very stuff of pipe dreams.

● A piece of information from Sir John Hill which was immediately seized upon by the media was the news that Harwell had re-started a programme to examine the possibility of disposing of dangerous radioactive waste in geological strata and the ocean deeps. Sir John regretted that he could not say where the Harwell men were looking or when the UKAEA might think of dumping; it was all very much a general enquiry at the moment. Quite coincidentally, the Natural Environment Research Council (NERC) announced last week that Britain was to pay \$1 million a year to join in the International Phase of Ocean Drilling (IPOD) of the Deep Sea Drilling Project (DSDP).

The term "International Phase of Ocean Drilling" is in fact a euphemism for a phase which might more accurately have been called the survival-by-a-hair's-breadth period. The DSDP, which has been supported almost unilaterally by the USA since its inception

In a controversial report published this week, a committee of the National Academy of Sciences (NAS) has convincingly demonstrated how little we know about the potential long term consequences of a full-scale nuclear war. The committee attempted to assess the magnitude of physical and biological damage in regions far removed from the target area, decades after a massive nuclear attack. Not surprisingly, it has suggested that there are huge areas of uncertainty—such as the possibility that a significant climatic change could be triggered—but it could predict no single effect serious enough to wipe out human life completely.

The report has raised considerable controversy because, aside from the emotional nature of the subject matter, it is open to a wide range of interpretations. Thus, Dr Philip Handler, President of the NAS, stated in a letter printed as an introduction to the report that the committee has concluded that *Homo sapiens* would survive the "horrendous calamity" of a massive nuclear exchange, while the Federation of American Scientists (FAS) suggested in a commentary on the report that the uncertainties in the calculations render such conclusions unwarranted.

The study, which was carried out for the Arms Control and Disarmament Agency (ACDA), took as its starting point a war resulting in the detonation of about 10,000 megatons of nuclear explosives in the Northern Hemisphere—equivalent to about half the destructive capacity of the world's nuclear arsenals. The committee concentrated its attention on phenomena likely to occur "at distances on the order of continental separations from the detonations", and it made no attempt to analyse possible economic and political consequences from such a holocaust. Findings include:

- Perhaps the most significant worldwide effect may result, not from radioactive fallout, but from massive destruction of the ozone layer. The committee notes that a major nuclear

exchange would inject huge amounts of nitric oxide into the stratosphere, which in turn could destroy between 30 and 70% of the ozone layer in the Northern Hemisphere, and about 20–40% of the layer in the Southern Hemisphere. Although much of that destruction would be repaired by natural processes in three or four

After the Third World War

by Colin Norman, Washington

years, it may take as long as 40 years for the ozone layer to be restored completely, the committee reckons.

The consequences of such an event would be a very large increase in the amount of ultraviolet radiation reaching the Earth's surface; this would damage plant life and present a severe health hazard to animals, including man. The committee notes, for example, that increased exposure to ultraviolet radiation "might have a significant impact on a great variety of [plant] species . . . and possibly could have serious implications for the ecosystem of which (particularly sensitive species) are a part". As for food crops, the committee suggests that plants such as peas and onions could be killed by large increases in ultraviolet radiation. The effect on man would be to increase the incidence of skin cancer in mid-latitudes by up to 30%, and "incapacitating cases of sunburn in the temperate zones and snow blindness in northern countries" would be expected.

- Radioactive fallout in the Northern Hemisphere would average about 1 Ci km⁻², but there would probably be "hot spots" where the fallout could be an order or magnitude greater. The committee states that "there would be no widespread effect" on plants from fallout, but in the hot spots, "minimum damage to ecosystems dominated

by radio-sensitive plants might occur". Some foods may become contaminated at levels "approaching the upper limits of present standards", however.

The effect on animals would, on the other hand, be more pronounced. An increase of 2% in the incidence of cancer would be likely, and a similar increase in the incidence of genetic disease would also result.

- As for effects on climate, the committee notes that a large nuclear exchange would inject vast amounts of dust into the atmosphere, which would reduce the amount of solar radiation reaching the Earth and possibly reduce global temperatures as a result. Moreover, destruction of a large part of the ozone layer might also lead to a reduction in temperatures. Even small changes would have serious implications for crop production, and the committee notes that "substantial changes in weather extremes . . . which could be of major importance to agriculture, have also been related plausibly to small changes in global mean climate". The committee said, moreover, that it could not rule out the possibility that a small perturbation in global temperatures "also might lead to major global climatic changes".

What are the implications of such findings for policymaking? Opinions vary. According to Dr Fred Iklé, Director of the ACDA, the committee's findings underline the futility of all-out nuclear war, because they suggest that there may be a serious ecological backlash from a massive nuclear attack.

But the FAS suggested in its statement that the conclusion that mankind might survive a nuclear holocaust has little relevance to public policy.

The committee should have recommended getting rid of all US and Soviet nuclear bombers, which would eliminate about 80% of the world's nuclear megatonnage, thereby reducing potential long-term effects of nuclear war, says FAS. □

in 1968, was expected to cost \$14 million in the present fiscal year, and the chances are that it would have collapsed in a bankrupt heap if five foreign governments hadn't agreed to bail out the project. Very broad hints were dropped during hearings of the House Appropriations Sub-committee last February that Congress might only look favourably on requests for more funds if an international interest could be demonstrated—and demonstrated in dollars and cents. The Soviet Union and Japan have also signed up for \$1 million worth of interest, West Germany is renegotiating an involvement

of two years' standing, and France is also expected to buy in at the going rate, leaving the USA to find the remaining \$9 million.

Since the information gathered by the DSDP in its pre-international phase was fairly readily available to friendly nations at something less than \$1 million a year, it seems reasonable to ask why the UK should bother laying out money. The answer is that if the UK (and the other four volunteers) hadn't, then there wouldn't have been any further information to receive at bargain rates or any other: that the UK would now have immediate access

to results, but that the emphasis in British participation was chiefly on the economic and technological benefits to be gained. This is meant to mean that the UK will be in a better position to exploit seabed resources (if and when the good ship *Glomar Challenger* happens on any) and hard scientific know-how, and that the exercise will develop the nation's deep-sea technology experience, presumably to the benefit of North Sea oil operations. Perhaps it will also provide answers to interesting questions like: "Where is there a nice spot to dump a ton of radioactive waste?" □

TO irradiate or not? That is the question. The issue involved is simple: is irradiated food—in this case, wheat—safe for human consumption? A year-old row over the matter between two premier research institutions, one of which belongs to the Department of Atomic Energy (DAE) and the other to the Ministry of Health, has recently burst into the open. And as wheat is the staple diet of much of the Indian population, the question has assumed enormous significance.

Work on the radiation preservation of foods has been going on at the DAE's Bhabha Atomic Research Centre (BARC) in Bombay for quite some time. The BARC has long been a proponent of the radiation method and has been urging the authorities to grant health clearance to specific low-dose food irradiation processes.

"Chemical disinfestation methods such as fumigation require repeated application because they do not eliminate insect eggs. They may also leave harmful residues in the treated grain. Irradiation, on the other hand, is a one-shot process that completely kills or sterilises the common grain pests, their pupae, larvae and even the eggs deposited inside the grains." Commending the radiation method thus, Director of the BARC, R. Ramanna, said in a lecture at a Bangalore college last year: "The work carried out at the BARC, especially on wheat, potatoes and onions, clearly shows that preservation of foods by radiation is not only economic but safe according to all standards; the earlier we adopt this method the better it is".

But the National Institute of Nutrition (NIN) in Hyderabad has stated (in its annual report for 1974) that irradiated wheat could be hazardous to health. Specifically, it claims to have found that: (a) consumption of irradiated wheat by both animals and humans caused them to develop polyploidy—a condition characterised by more than the normal number of chromosomes in cells; (b) rats which were fed on irradiated wheat and which developed abnormal or polyploid cells transmitted these chromosomal abnormalities to their offspring; (c) mutations took place faster in animals that ate irradiated wheat; (d) there was a significant reduction in reproductive cells in malnourished rats on irradiated wheat diet; and (e) incidence of polyploidy was reduced if irradiated wheat was stored for 12 to 14 weeks before consumption.

As soon as these findings were made public the DAE responded by repudiating the NIN claim. It maintained that its own experiments (at the BARC) had not revealed any of the 'hazards' referred to by the NIN. In support, the DAE statement mentioned that, on the

basis of similar feeding trials with laboratory animals, several countries (including the USA, the USSR, Canada, France, Holland and Denmark) had declared a variety of irradiated foods as safe and wholesome for unlimited human consumption. Moreover, specialised UN agencies like the Food and Agriculture Organisation (FAO) and the World Health Organisation (WHO), it said, had rejected contentions that irradiated wheat was meant exclusively for animals and not for human beings. The DAE also refuted the charge that it had "already spent crores" in setting up laboratories

Row over irradiation of wheat

from Narender K. Sehgal, Jullundur

and equipment for radiation preservation of food.

Following this the NIN repeated its earlier warning about hazards from irradiated wheat and answered criticism of its findings. It said it had experimented with children only after "totally accepting" the DAE claim that its studies had shown irradiated wheat to be "harmless". (Although irradiated wheat was earlier found by the NIN to cause polyploidy it went ahead and fed this diet to five malnourished young children aged two to five for six weeks. This attracted severe criticism from many quarters.) But, "when we fed children irradiated wheat and found abnormal cells in circulation the study was promptly terminated", the statement added.

The DAE observation that polyploid cells in fact occurred naturally in humans and animals was countered by pointing out that in its (NIN's) experiments "feeding of irradiated wheat was consistently associated with a four- to ten-fold increase in the number of polyploid cells"; this was found to be true in rats, mice and monkeys irrespective of the protein content of the diet or the age of the animal. Although conceding that the precise significance of increased polyploidy was perhaps something that could be debated, the NIN statement pointed out that this condition had been generally associated with a kind of cancer.

The NIN denied the DAE charge that it had either underplayed or tried to conceal its own finding that the 'hazards' mentioned by it were absent when irradiated wheat was stored for over 12 weeks before consumption. But, the statement said, the NIN studies had further shown that the production of aflatoxin poison was considerably greater in stored irradiated wheat and potatoes than in the corresponding unirradiated foods. The NIN

also noted that, in the case of onions and potatoes, rotting rather than sprouting was a major problem in India and that irradiation seemed to accelerate rotting even though it did stop sprouting. (This observation was in reference to the fact that the BARC had been devoting considerable attention to preventing sprouting in onions and potatoes, instead of concentrating on the problem of rotting.)

The NIN statement said in the end that it did not look upon the matter as a prestige issue; "we are always prepared to look in all objectivity at any new evidence which may throw an entirely new light on the problem".

While the two institutions continue to exchange charges and arguments over apparently conflicting results, an expert technical committee has called for further "joint studies by the DAE and the NIN on post-irradiation storage problems of irradiated wheat and potatoes". This committee, headed by Dr M. S. Swaminathan (Director-General of the Indian Council of Agricultural Research), was set up last year at the instigation of the Prime Minister to go into the whole matter of the safety of irradiated wheat, onions and potatoes.

The committee, in its report submitted recently to the Ministry of Health, has steered clear of the safety question. In view of the problems of aflatoxin production during storage of wheat and potatoes (in the latter case, rotting as well), the government, the committee said, could consider clearing irradiated wheat and potatoes for sale only after insisting on proper storage—a minimum of six months for wheat and four months for potatoes.

The committee based its report on studies in India on wheat and on data from other countries in the case of potatoes, since none was available from India. In the case of onions, no studies had been carried out in India and the committee were of the view that studies abroad were "not yet conclusive about the safety of irradiated onions for human consumption". So it declined to make a recommendation at this stage.

In view of the serious differences between the NIN and the DAE over irradiated wheat, the Swaminathan committee called for an expert evaluation of the data by a team consisting of a statistical expert from the Maharashtra Association for Cultivation of Science and a geneticist from the Jawaharlal Nehru University. The committee also suggested that an inter-ministerial technical group should examine the techno-economic questions, the cost-benefit analyses and the feasibility of adopting radiation technology for food preservation on a large scale. □

ON August 10, on the beach at Cape Canaveral in Florida, I saw a red-headed man, sunburned to look like a boiled lobster, applying novocaine cream to his glowing back. The only unusual circumstance was that the man was Mike McElroy, whose field is the physics and chemistry of planetary atmospheres and who has loudly warned us against the ultraviolet perils of destroying the ozone layer by using aerosol spray cans. Aha, I thought: one foot in the sea, and one on the shore—to one thing constant never. For surely he, of all people, should have kept his shirt on.

The proposed ban on aerosol spray cans is a model of scientific virtue. It will save the sacred Environment of our fragile space-ship Earth from the excesses of rampant technology. It will prevent cancer. It will enable academic atmospheric physicists to cover themselves with glory—their abstruse field is Being Put To Use, while visions of research grants dance through their heads. Investigation of the subject is “a natural” for the techniques, instrumentation and vehicles of the space programme, and so it will be a first-rate spin-off for NASA. The ban may even save some metal from being used in cans, and it will benevolently force people to use their flabby muscles in wielding paint brushes. The New York Subway System will perhaps be able to clean up its cars, which are now hideously festooned with paint applied from spray cans by the vandals who also deface rocks by the roadside. Spray-on armpit deodorants will be replaced by the roll-on type, and the consumer will pay the same astronomical mark-up for a cent’s worth of some aluminium salt to close the axillary sweat glands. Furthermore, no

Spray no more, ladies



THOMAS H. JUKES

one will ever be able to prove the ban was not needed—there is no neighbouring planet that could be used as a control. Private industry doesn’t have a chance—the public got along very well before aerosol spray cans were introduced, and the task of the courts in denying appeals against a ban is made childishly simple by the magic word “cancer”.

Indeed the National Resources Defense Council has intoned “For every year that the banning of these aerosols is delayed, evidence shows that an additional 2,000 to 4,000 people will be stricken with skin cancer”. (Note the expletive use of the word “stricken”.) What evidence shows this, I wonder? Ultraviolet light has been reaching the skin ever since human beings first appeared. How many cases of skin cancer are caused by sunlight; what is the incidence at different latitudes; and how is it related to the percentage of the body surface that is exposed? Someone must have done some extrapolating to come up with the figure of 2,000 to 4,000 people, especially when

the effect of aerosols on the ozone layer is a matter of debate rather than measurement. And then, of course, ultraviolet light protects against rickets. . . . If the danger of cancer from solar ultraviolet light is so great, why not suggest that people keep their clothes on? From Sydney to Stockholm, the near-naked and naked white-skinned legions loll on beaches, seeking to acquire the shadowed livery of the burnished sun, and to hell with skin cancer.

A few cynical sceptics remain unconvinced. Professor Richard Scorer says it is Much Ado About Nothing. He has been rude enough to say that the talk of the threat to the ozone layer is a publicity gimmick concocted by doomsayers using scare tactics to gain headlines, and that most of the organic chlorine compounds now measured in the stratosphere are of natural origin, or from the deliberate burning of vegetation. Personally, I rather favour his thesis that the environment is full of checks and balances, and “is not as frail as some people picture it”. But heaven forbid that I should suggest that a large amount of research on this latest scare is not needed; it will train young scientists, and it will help to restore badly needed public confidence in the social values of research in the natural sciences.

And perhaps—who knows?—we may eventually be able to ride on the New York Subway, and read the advertisements for deodorants unmarred by paint from aerosol spray cans! But I am afraid that I underestimate the ingenuity of industrial chemists, who undoubtedly are hard at work to devise new spray propellants that do not destroy ozone, thus converting all our sounds of woe to hey-nonnny-nonnny.

THE first protocol of the Anglo-Soviet Joint Commission on Health Cooperation, signed on September 25, 1975 by Mrs Barbara Castle, Secretary of State for the Social Services, and Professor Boris Petrovskii, Soviet Minister of Health, represents the first stage in the implementation of the Anglo-Soviet Agreement on Cooperation in Medicine and Public Health which resulted from Mr Harold Wilson’s visit to Moscow last February.

The agreement itself is a wide ranging document, dealing in general terms with “exchanges of information on new equipment, pharmaceutical products and technological developments related to medicine and public health,” reciprocal health-service arrangements for British and Soviet citizens requiring immediate medical treatment while in the other country, and the elaboration of (so far unspecified) programmes of

Healthy exchanges

from Vera Rich, London

joint research. Only one section—Article 2A—names any specific subjects for cooperation, and it is precisely these, influenza and other communicable diseases, ophthalmic diseases, and the organisation of medical care for traumatic and cardiac emergencies, which are the subject of the present Protocol.

Under the terms of the Protocol up to 25 man-months during the first year of its operation may be spent by each side on research in the other’s country, with due arrangements made for the supervision of research and the pooling of results. The institutions involved in the exchange will be the

Institute of Ophthalmology of the University of London and the All-Union Institute of Ophthalmic Diseases; the Public Health Laboratory Service, Colindale, and the Moscow Institute for Virus Preparations; and the Medical Research Council Accident and Burns Unit, Birmingham, and the Moscow Institute for Emergency Medical Services.

Although the agreement makes provision for sending patients for treatment from one country to the other the Protocol stresses that in view of the “high level of development” of both health services, such exchanges are envisaged in exceptional circumstances only, and it seems likely that cases such as that of the Russian baby Irina Chudnovskaya, who has already been treated at the Brompton Hospital for a heart condition, will remain relatively rare. □

correspondence

Radon emissions

SIR,—Wendy Barnaby (August 28) writes on the problem of radon emission from the tailings of uranium milling in Sweden. This problem would arise from the large volume of uranium shale that has to be treated. She describes Professor Robert O. Pohl's report that "radon can escape more easily from the broken ground of a mine than from an undisturbed terrain". This report is well known among Swedish experts. Thorium-230, a metallic chemical element, and radon-222, a noble gas formed from uranium, can be found wherever there is uranium in the Earth's crust—not only in uranium ores and mines but also in primary rock.

In 1965, the Swedish Atomic Energy Company (AB Atomenergi) started operations at Ranstad where there is a large uranium deposit. From the very beginning, the emission of radon from the leaching residues has been studied. It has been shown that the emission from recently deposited residues is approximately equal to that measured in the open pit on the uranium shale bench. It has also been shown that the emission of radon from the restored tailings is of the same order of magnitude as that from the undisturbed terrain within the open pit area. In Ranstad, large scale tests have been made during the past few years on the deposition of leaching residues. The residues were laid out in stockpiles after mixing with fine-ground limestone and packed with a vibrating roller. After that, the area was covered with earth from the open pit and seeded.

As well as giving the landscape an attractive finish and making it possible to use the ground for agricultural purpose, the emission of radon has been minimised.

SVEN-ERIC BRUNNSJO

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Stockholm, Sweden

Cancer at work

SIR,—Your article "Cancer at work" (September 18) is admirable in that it draws attention to environmental carcinogens. It is regrettable, however, that no mention is made of the painstaking work of S. A. Henry of the Medical Inspectorate of Factories in finally convincing authorities of the

risks of tar and in particular of lubricating oils used outside the cotton industries (*Cancer of the Scrotum in Relation to Occupation*, Oxford University Press, 1946). The considerable emphasis laid on the historical background leading to the recognition of these carcinogens does, moreover, distract attention from the fact that these problems are still with us. The sentence "Although less common, scrotal cancer still occurs in industry in conditions in which men are exposed to lubricating oils" is a gross understatement of the present situation. Although there are geographical variations in the occurrence of lubricating-oil cancer which are as yet unexplained, the incidence in the West Midlands not only of scrotal cancer but of skin cancer and possibly of lung cancer is a cause for considerable concern.

C. N. D. CRUICKSHANK

University of Birmingham, UK

Leprosy treatment

SIR,—Being engaged in leprosy control work in India, I daily face exactly those problems mentioned by Browne and Davey (May 15) as the main obstacles in implementing regular and sustained treatment of leprosy patients, namely, the social aspects of leprosy and the patients' attitude to leprosy.

I would like to add one more human factor and that is the importance of motivated staff. Much has been written about planning, education of the public and the approach of the leprosy patient, but little or no attention is usually paid to the field worker. Yet he is the key person in the strategy for leprosy. It is not lack of planning or of effective drugs or of a vaccine which has caused so many leprosy control programmes to founder but lack of dedicated and competent field staff. So our first concern must be to give field workers the right motivation.

My second point concerns the treatment of leprosy. Both Browne and Davey, and Crawford (March 20), refer to sulphones only but nowadays no leprosy control unit can function properly unless it has Lampréne at its disposal as well. The absolute indication for Lampréne is resistance against Dapsone. This was very rare, but according to Browne and Davey "it is not now, and the recent figures coming from countries where the problem has

been adequately investigated are very disturbing to say the least."

The greatest advantage of Lampréne, however, is that it has both bacteriostatic and anti-inflammatory properties. Thus treatment of Lampréne can be continued during reactions, whereas treatment with Dapsone is often discontinued during reactions, either by the doctor or by the patient himself. This may ultimately lead to resistance against Dapsone in lepromatous patients who are prone to reactions. It is for this momentous group of patients that Lampréne is absolutely indispensable. In Dichpalli, with approximately 10,000 leprosy patients receiving treatment, 2 to 3% require Lampréne and the proportion is still increasing.

L. M. HOGERZEIL

Dichpalli, India

Czech conference

SIR,—I recently registered as a participant in the International Conference on Low Radioactivity Measurement and Applications (LRM) in Czechoslovakia (October 6–10,) paid for the conference fee, and got written acceptance of my participation and hotel lodgings. Also a visa for that particular conference I received from the Czechoslovakian Embassy in Bonn.

On September 17, I received a letter from the organiser (also in Czechoslovakia) to say that I would not obtain a visa to attend the LRM conference due to "news from the Czechoslovak ministry of Foreign affairs." The Bonn Czechoslovakian embassy told me on demand that the validity of my visa could by no means depend on such a letter, and that I might still travel there.

Later I received a telegram from the organiser telling me in a harsh tone and without giving reasons that "we cannot accept your participation in the conference."

I never had any political or other differences with my own or foreign countries, but I would not think of participating in this conference after such an incredible sequence of events. May I suggest to Czechoslovakian politicians: If you really want to build up effective scientific research why not treat your scientists better and their guests in a less arbitrary manner?

J. C. FREUNDLICH

Cologne, FDR

news and views

It is a long time since the discovery of bacterial transformation demonstrated that small amounts of foreign genetic information can be introduced into a living cell, and can function there. The explosive development of techniques for genetic and general biological experiments on eukaryotic cells in culture has led naturally to the search for an analogous system for the transfer of genetic information between cells of higher organisms.

Quite substantial progress has been made at several different levels, and it is now clear that the genetic information of complex organisms can be introduced into a foreign cell, and can function satisfactorily in this new environment. Whole nuclei can be introduced mechanically into the enucleated cytoplasm of another cell type (Gurdon, *Adv. Morphogenesis*, 4, 1; 1964). Somatic cell hybrids, usually made with inactivated Sendai virus (Harris, *Cell Fusion*, Oxford University Press, 1970; Ephrussi, *Hybridization of Somatic Cells*, Princeton University Press, 1972), initially also produce two or more different functional nuclei within one cytoplasm, but this is followed by nuclear fusion resulting in mononucleate cells containing chromosomes of both parental types. In many situations, particularly where the cells used are from different species, these hybrids subsequently lose chromosomes of one parental type, leading eventually to cell lines which contain relatively few 'foreign' chromosomes within their nuclei. Chromosomes introduced in this way can express their genetic information, and this system has been extensively used for the formal genetic analysis of human chromosomes (*Rotterdam Conference*, The National Foundation, 1974), studies on regulation of gene expression and so on.

An extreme example of chromosomal segregation in hybrid cells is provided by situations in which an enzymatic function of one parental type is retained in the hybrid, but no corresponding chromosome can be detected in the karyotype (Schwartz *et al.*, *Nature new Biol.*, 230, 5; 1971; Klinger and Shin, *Proc. natn. Acad. Sci. U.S.A.*, 71, 1398; 1974). The probable explanation is that a chromosomal rearrangement has occurred, translocating a small segment of the donor genome into the host cell's karyotype. Thus, a microscopically undetectable amount of foreign genetic

Information transfer in mammalian cells

from M. Bobrow and E. Solomon

information is integrated, with some degree of stability, into the host cell genome.

Many workers have attempted to demonstrate the uptake of isolated chromosomes or purified DNA by eukaryotic cells. Only recently, however, have any persuasive examples of this type of phenomenon been described. McBride and Ozer (*Proc. natn. Acad. Sci. U.S.A.*, 70, 1258; 1973) incubated isolated Chinese hamster chromosomes with mouse fibroblasts deficient in the enzyme HPRT. The absence of this enzyme makes cells unable to grow in selective medium containing aminopterin. In such a medium, McBride and Ozer isolated cell lines which had acquired the hamster HPRT enzyme, thus correcting the genetic deficiency of the mouse cells. Similar results have been obtained on incubating mouse cells with isolated human chromosomes (Burch and McBride, *Proc. natn. Acad. Sci. U.S.A.*, 72, 1797; 1975; Willecke and Ruddle, *Proc. natn. Acad. Sci. U.S.A.*, 72, 1792; 1975).

The difficulty in experiments of this sort is to distinguish genuine transfer of genetic information from mutational or induced changes occurring in the host cells themselves. Most enzyme-deficient cell lines used in selective culture systems do have a low but detectable rate of spontaneous reversion to the enzyme-producing phenotype. Results such as those of McBride and Ozer are made credible by the great care taken in characterising the HPRT enzyme in the 'corrected' cells, by a variety of techniques, as being of donor and not of host origin.

In none of these cases was there any identifiable donor chromosome present

in the karyotype of the derived cell lines. Some of these 'corrected' mouse cell lines are quite stable in culture, and are possibly again the result of small chromosomal rearrangements, with fragments of donor chromosome being inserted into the host karyotype. The size of fragment transferred must, in all these experiments, be pretty small (estimated as less than 1.5% of the human genome) as no donor enzymes have been detected in the resultant cell lines other than the one (HPRT) specifically selected for by the culture system. The reason for the integrated fragment being so small is not yet apparent. This unfortunately limits the use of the technique for genetic experiments other than those dealing with a selectable enzyme itself, or genes very closely linked to it. □



A hundred years ago

Oceanic Circulation

MR. CROLL'S statement (vol. xii, p.494), that the North Atlantic in lat. 38° is above the level of the equator, is based partly on the *Challenger* soundings and partly on Muncke's determinations of the thermal expansion of sea-water, which, however, were not made on sea-water at all, but on a saline solution prepared for him by Leopold Gmelin, according to data furnished by the incomplete analyses of Vogel and Bouillon La Grange. As Mr. Croll's statement depends on such very minute differences of volume, I am led to ask him to compare the rate of expansion of *real* sea-water, as determined by Prof. Hubbard, with Muncke's table; he will notice a discrepancy sufficiently wide to make it a matter of interest to ascertain how far the employment of the American observations may serve to substantiate or modify his conclusion.

G. E. THORPE

Yorkshire College of Science,
Oct. 11.

from *Nature*, 12, 514; October 14, 1875

EVERY schoolboy is told that gravity is only attractive. Unlike selenite—that amazing substance written into science fiction by H. G. Wells—ordinary matter always falls towards other matter.

This asymmetric property of gravity used to pose a problem to inventors of model universes. What stops everything in the Universe from falling together at one place? During the adolescence of his theory of general relativity, Einstein applied his newly proposed gravitational field equations to a simple model universe. He was dismayed to find that this universe would not stay still—it had either to collapse or expand. Interpreting this circumstance as a defect of the theory, he set about tampering with the field equations to put the matter right. He was able to add an extra term to the equations, without compromising the basic physical principles of the theory, which did, indeed, enable a static model universe to be constructed. The result was a novel curiosity—a universe which was finite in volume, yet unbounded; a consequence of the curvature of space actually closing the universe up on itself. Included in the extra term is the ‘cosmological constant’.

The physical effect of this extra term is to introduce a repulsion in the gravitational action between distant matter. This repulsion is negligible over terrestrial distances, but can dominate on a cosmic scale. By doing a balancing act between ‘ordinary’ gravitational attraction and cosmic repulsion, Einstein’s model universe could remain static.

By a quirk of fate, scarcely had the

Is the Universe running away with itself?

from P. C. W. Davies

model been constructed than it was discovered by Hubble that the Universe was not static anyway, but in a state of general expansion. New models of this expanding Universe had already been constructed by Friedmann, and the cosmological constant was not needed, which was a relief to the physicists, who naturally regarded this term as rather contrived. Since then, most cosmologists have been happy to drop the extra term (although it has been resurrected on a number of occasions, principally because it can enable the Universe to be somewhat older than its expansion rate suggests, by delaying in a ‘nearly static’ phase). This philosophy has long fitted in nicely with observation, which has shown the cosmological expansion rate steadily slowing down with time. Such a deceleration is explained naturally by the supposition that the galaxies are pulling back on each other as they move apart. If there was cosmic repulsion, then the galaxies ought to be accelerating not decelerating. A simple calculation shows that this acceleration would actually be exponential—a cosmic runaway motion in which the Universe would for ever expand faster and faster.

During the past year, a major rethink

by astronomers has led to the suggestion that the Universe may be doing just that. This remarkable conclusion is the result of combining new observational data with theoretical calculations about a range of different astronomical studies. These include not only direct measurements of the cosmic deceleration, but information about the density of matter in the Universe and the ages of galaxies. Although all of these are subject to many uncertainties and errors, cumulative evidence has led two astronomers to claim that an accelerating universe is the most plausible model. In this issue of *Nature* (page 454), James E. Gunn (Hale Observatory) and Beatrice M. Tinsley (Lick Observatory) present their case. Although they are mindful of the somewhat shabby history of the cosmological term in general relativity (“most relativists find it repulsive on principle rather than by observation”) they conclude that it is probably non-zero. Collecting together all the various constraints on the cosmological parameters leads them to suggest not only that the Universe is in runaway motion, but that it is also spatially closed, like Einstein’s original model.

Naturally it will await new data and the shedding of well entrenched ideas before this model of the cosmos becomes a serious contender. If this should prove to be the case then the time will have come for the physicists to take a new look at the unwelcome appendage to Einstein’s general relativity and try to understand fully the nature of the mysterious cosmic repulsion.

Sir Charles Lyell

from Martin J. S. Rudwick

The Charles Lyell Centenary Symposium sponsored by the Royal Society, the Geological Society and the British Society for the History of Science was held in London on September 1–5. It also formed the sixth symposium of the International Committee on the History of the Geological Sciences (INHIGEO).

THE name of Sir Charles Lyell (1797–1875) must be familiar to every geologist, but few perhaps have actually read his classic work on the *Principles of Geology*. Likewise historians of science often refer in passing to Lyell’s work as a preparation for Darwin’s, without perhaps understanding fully its importance in its own right. The Charles Lyell Centenary Symposium was designed to bring geologists and historians together for

five days of intensive discussion, not only about Lyell himself, but also about his antecedents in geological science, his contemporaries and his legacy to subsequent work.

As is usual when historians of science and working scientists meet, there was at times some tension between those who see the broader historical context as all-important, and those with greater interest in the content of the scientific ideas and observations of the period. But this mingling of professional historians with special expertise in the cultural climate of 19th century science, and geologists who often have a more detailed knowledge of particular scientific problems, was generally felt to be an enriching experience on both sides. A more interesting and more important cleavage of opinion emerged during the symposium, cutting across these lines of professional affiliation. This concerned the nature and even the reality of whatever ‘revolution’ or ‘paradigm’ Lyell may be claimed to have initiated

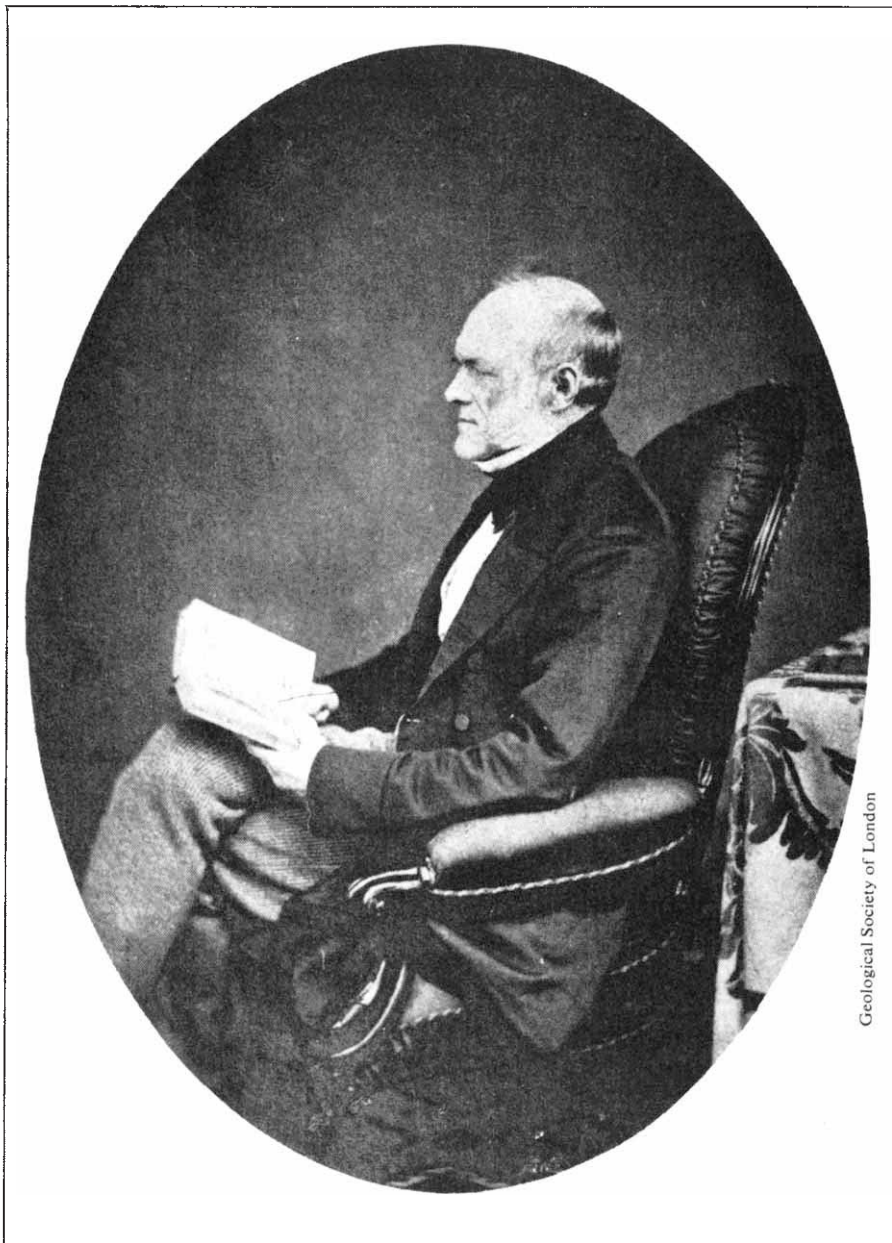
in geology. This is a topic that has been keenly debated among historians of geology in the past few years, but which has not yet penetrated much beyond the specialist literature.

After an inaugural speech by Sir Kingsley Dunham (Institute of Geological Sciences, London), the symposium was plunged at once into the heart of this controversy by a paper urging the members to apply Lyell’s own scientific principles to the historical interpretation of Lyell himself! Using Lyell’s own re-writing of the history of geology as his theme, R. S. Porter (Cambridge University) urged the symposium to be ‘uniformitarian’ about 19th century geologists, and to reject ‘catastrophist’ historical accounts that see Lyell’s work springing miraculously out of an earlier morass of obscurantism. Several later speakers took up this challenge, enlarging on Lyell’s own view of his place in the development of geology, and relating his work to that of his predecessors and contemporaries in

Britain, Sweden, Germany, Italy, France and elsewhere.

The view that there was a sharp disjunction between Lyell and his 'catastrophist' opponents was re-stated by Lyell's most recent biographer, L. G. Wilson (University of Minnesota), but without the conceptual comparison that might have led to a fuller discussion of the precise nature of Lyell's originality. Indeed, several speakers emphasised how the different meanings of 'uniformity' in geology had often been confused by Lyell himself, and had led to continuing misunderstandings in geological debate up to the present day. As C. J. Schneer (University of New Hampshire) remarked in his final summing up, what emerged from the symposium was very much a picture of Lyell 'warts and all'—a man who could be less than generous in acknowledging publicly his debts to other scientists, and whose work was perhaps less 'revolutionary' than he and his more enthusiastic admirers believed; and yet this more balanced and human picture of the man could hardly detract from our appreciation of his achievement in helping to consolidate the methods and goals of his chosen science. J. D. Burchfield (Northern Illinois University) summarised the way in which Lyell's cherished theory of a 'steady-state' earth crumbled in the late 19th century before the onslaughts of the physicists led by Lord Kelvin; but other speakers emphasised the continuing impact that Lyell's more general approach to geology had had in regions as far apart as Germany, the USA, Japan and the Pacific.

In addition to the main sessions at Imperial College, the symposium members were able to imbibe something of the atmosphere of the scientific institutions of Lyell's day, with visits to King's College and University College London, the Geological Society, the Geological Survey (as it then was), the British Museum (Natural History) and the University of Oxford, at each of which an exhibit related to the theme of the symposium was on display. In the course of these visits, a paper by J. B. Morrell (University of Bradford) showed how Lyell's own choice of affiliations to such institutions reflected their relative value to the rising professionalism of the science of that period; while M. J. S. Rudwick (Vrije Universiteit, Amsterdam) argued that visual modes of communication in published works on geology had played a decisive role in the emergence of the science. For many members, however, the high point of the symposium was the visual re-appearance of Lyell himself (ably impersonated by the symposium secretary, J. C. Thackray of the Institute



Geological Society of London

of Geological Sciences) in the lecture theatre of the Royal Institution, where a re-enactment of extracts from his early public lectures gave a vivid demonstration of his persuasive rhetoric.

Geology is a science in which the impact of first-hand experience of particular phenomena has often been deeply influential on the theoretical development of individuals. It was therefore appropriate that the symposium included two field trips, before and after the main meeting, to classic geological areas that were closely associated with Lyell: the Isle of Wight and the New Forest, where he spent his childhood and early manhood, and eastern Scotland from Berwickshire to Angus, where he was born and where he later did some of his best early research. Here perhaps the high point was the party's reception by Lady Lyell and Lord Lyell at the family home of Kinnordy House,

where books and papers from Charles Lyell's library were on display.

The symposium was highly successful in re-creating the historical context in which Lyell and his contemporaries strove to make geology a science with firmly established methods and clearly defined cognitive goals; and the widespread interest of geologists in this achievement is surely evidence of its continuing influence in the science. The superbly smooth running of the symposium, due chiefly to the chairman of the organising committee, D. A. Bassett (National Museum of Wales, Aberystwyth), might seem to make any criticism ungracious. But as R. Hooykaas (Rijksuniversiteit Utrecht and Vice-President (Europe) of INHIGEO) pointed out in his final vote of thanks, if English is to be used—as it is nowadays—as the common language of science and scholarship at international meetings, those

who have English as their native tongue must learn to be more considerate towards those who are less fortunate in this respect. As at other such conferences, too many papers by British and American speakers were delivered at ultra-high velocity and with low signal-to-noise ratio. Such thoughtlessness is hardly fair on the rest of the world. $\square$

Alpha-clustering in nuclei

from P. E. Hodgson

THERE are now very many lines of evidence that show that the nucleons inside the nucleus tend to form clusters that persist long enough to affect many nuclear properties. The most likely form of cluster is the alpha particle because of its high symmetry and binding energy, and much evidence for the presence of alpha clusters comes from nuclear structure calculations and from measurements of the cross sections of many nuclear reactions.

The structure calculations of Brink and Castro (*Nature*, **248**, 280; 1974) showed that when nuclear matter is reduced to about one-third of its density at the centre of a heavy nucleus it tends to coalesce into alpha clusters. This suggests that in the outer regions of the nucleus, where the density is low, it is very likely that alpha clusters will be found.

In the case of light nuclei the Hartree-Fock calculations of the nuclear density show very directly the regions of maximum density, three for carbon, four for oxygen, five for neon and so on, and this suggests that these nuclei are made up of clusters of nucleons resembling alpha particles.

The familiar process of alpha decay is a very direct indication that alpha particles are to be found in nuclei. Theories have been developed that express the probability of alpha decay as the product of one factor representing the probability of an alpha particle being formed on the nuclear surface and a second factor giving the probability that it will tunnel through the potential barrier and be emitted. These theories are able to account for the very wide range of alpha decay lifetimes that occur in natural and artificial radioactive isotopes.

Many nuclear reactions also provide direct evidence of alpha clusters in nuclei. Among the most widely studied are the alpha-transfer reactions, in which an alpha particle is either removed from or added to the nucleus. Many such reactions have been studied, such as the ($^6\text{Li}, d$), ($^7\text{Li}, t$) and ($^{16}\text{O}, ^{12}\text{C}$) reactions and their inverses (*Nature*, **252**, 270; 1974). The cross sections of these reactions can be calculated by the same distorted wave theory as the one-nucleon transfer reactions by treating the transferred

particle as a single entity. On the whole this theory is very successful and allows us to calculate the probabilities of alpha formation in various states. These are the alpha-particle spectroscopic factors, which can be compared with detailed nuclear structure calculations (*Nature*, **255**, 373; 1975).

Other nuclear reactions that tell us about alpha clusters are the ($\alpha, 2\alpha$) and ($p, p\alpha$) reactions in which an alpha particle or a proton knocks an alpha particle out of the nucleus, but these are not so easy to treat theoretically and so have not yet given very precise information.

The (p, α) and (n, α) reactions at medium energies can give useful information about alpha clusters, since they often have a component due to the pre-equilibrium process, and if this is analysed it gives an estimate of the preformation probability of the alpha clusters (*Nature*, **245**, 12; 1973).

Reactions caused by pions and kaons have also been used to probe alpha structure, and many studies at low and high energies have provided some indications that alpha particles are present in the nucleus, and can be knocked out in favourable circumstances (*Nature*, **248**, 105; 1974; **249**, 616; 1974; **254**, 561; 1975).

It is very useful to have a clearer model of the behaviour of these alpha particles in the nucleus. Of course we know that all the phenomena mentioned can, at least in principle, be accounted for entirely in terms of the nucleons, without explicit mention of alpha particles. But nevertheless it is useful to think in terms of alpha clusters since they form an obvious physical feature of the nucleus, and if we can find a theory expressed in terms of alpha particles it will certainly be very much simpler than the corresponding description in terms of nucleons alone. We have to pay for the simplicity by some loss of precision, but this may well be acceptable if it gives us a simple model yielding physical insight into many phenomena and a way of calculating many features to fair accuracy.

Such a model can easily be constructed by analogy with the simple nucleon shell model. It is notable that all the reaction phenomena that can be understood by the nucleon shell model, in particular the optical model analysis of elastic scattering and of the resonances due to coupling to inelastic channels as well as the nucleon transfer data, are paralleled by similar phenomena for alpha particles.

This suggests that alpha-clustering phenomena can be understood by grafting on to the nucleon shell model the possibility that nucleons in outer orbits can combine to form alpha particles having a life long enough for their influence to be felt. These orbits have the quantum numbers that can easily be calculated from those of the constituent

Table 1 Theoretical and experimental alpha widths for ^{16}O

$J\pi$	$\Gamma_{\alpha}^{\text{exp}}(\text{KeV})$	$\Gamma_{\alpha}^{\text{th}}(\text{KeV})$ K = O ⁺ band	$\bar{f}$ (fm)
4 ⁺	27	17.5	1.436
6 ⁺	125	238	1.432
8 ⁺	—	385	1.425
		K = O ⁻ band	
1 ⁻	510	675	1.536
3 ⁻	1200	1750	1.5575
5 ⁻	700	≈ 2000	1.539
7 ⁻	750	776	1.668

Data from Buck, Dover and Vary (1975).

nucleons and alpha transfers can take place to and from these alpha orbits just as nucleon transfers can take place to and from the nucleon orbits.

According to this model the alpha particles are bound by an alpha-core potential, whose eigenvalues and eigenfunctions give the centroid energies and wavefunctions of the alpha states, just as for the nucleons. It is simplest to assume that this potential has the Saxon-Woods form,

$$V(r) = V_0 / \{1 + \exp[(r-R)/a]\}$$

where R and a are radius and diffuseness parameters. Once these parameters are fixed the depth of the potential V_0 is determined by the alpha-transfer data. The only remaining parameter, the probability of alpha formulation, is given by the spectroscopic factor for alpha-transfer reactions. This parameter is the only one in the model that has nothing corresponding to it in the analogous nucleon case.

Many studies of nucleon transfer reactions (*Nature*, **245**, 74; 1973) showed that the nucleon potential determined from an overall analysis of many reactions varies in a systematic way from nucleus to nucleus, and preliminary analysis of alpha-transfer reactions showed that this is also the case for the alpha potentials. This model has been applied to the alpha particle resonant states in medium weight nuclei and it is found that the energies and ordering of the eigenstates are qualitatively in accord with experiment.

A detailed study of this model as applied to light nuclei has been made by Buck, Dover and Vary (*Phys. Rev.*, **C11**, 1803; 1975). They determined the alpha potential by folding the densities of the nuclear core and the alpha particle

$$V(r) = -(2\pi\hbar^2/M)f \int \rho_A(r-r')\rho_B(r')dr'$$

where f is a real depth parameter. The densities ρ_A and ρ_B were taken from electron scattering analyses. It turns out that this potential has several advantages over the Saxon-Woods potential; in particular it can reproduce the energies of the states of low-lying rotational bands with essentially the same depth parameter.

Many studies of alpha-transfer reactions

(*Nature*, **252**, 632; 1974) have shown that they preferentially populate the rotational bands in light nuclei. This strongly indicates that these states have core α structure, and it is therefore natural to interpret such states as eigenstates of the above potential.

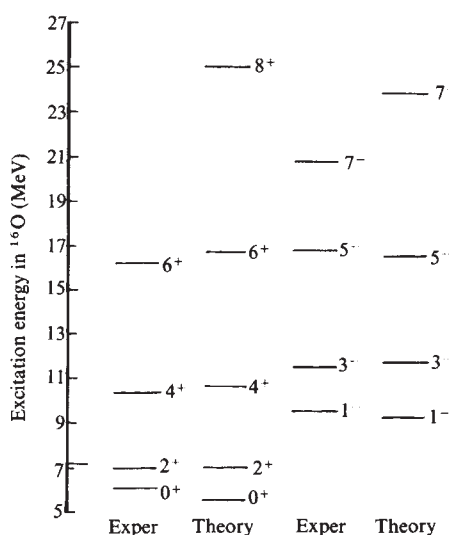
Buck *et al.* therefore applied their model to calculate the energy spectra of the $K^\pi = 0^+$ and 0^- bands in ^{16}O and ^{20}Ne , and the results for ^{16}O are shown in the figure. The parameter f was adjusted for each band, and the model then gives a good overall fit to the energies of all the states in that band. Since the widths are very strongly energy dependent, f was adjusted for each state to give the widths shown in Table 1. The required changes in f from the mean (band) values are only a few per cent. The correspondence between the measured and calculated widths indicates the qualitative reliability of the model. Since the calculated widths are very sensitive to the addition of an imaginary part to the depth parameter f , it is not correct to interpret as spectroscopic factors the ratios of experimental to calculated widths in this table.

The potentials give the alpha-cluster wavefunctions, and hence the alpha-core separations and root mean square (RMS) radii $\langle r^2 \rangle^{1/2}$ given for the ground state band of ^{20}Ne in Table 2. The values labelled $\rho^{(1)}$ and $\rho^{(2)}$ are obtained by Mosely and Fortune and by Arima and Yoshida respectively using Saxon-Woods potentials to generate the alpha-cluster wavefunctions, and $\rho^{(3)}$ is obtained from the Hartree-Fock calculations of Lee and Cusson. The model also gives values of $\langle r^2 \rangle^{1/2}$ that agree well with the empirical value of 2.9 fm given by Elton. All these models predict an anticentrifugal stretching effect. That is, the higher spin states correspond to a lower value of ρ and hence a smaller $\langle r^2 \rangle^{1/2}$. In the folding model this does not require a change in the radius of the potential itself.

As a final test of the model, Buck *et al.* calculate the electromagnetic transition rates between the states of the ground state band of ^{20}Ne , and these are compared

in Table 3 with the data and with shell model and rotational model calculations. In this calculation the effective charge was adjusted to $e_{\text{eff}} = 1.245e$ and gives values of $B(E2)$ quite close to those of the complete shell-model calculation which used $e_{\text{eff}} = 1.583$.

These results show that the alpha cluster model is able to give a good overall account of the energies and widths of the states of ^{16}O and ^{20}Ne , and the alpha-core separations, RMS radii and electromagnetic transition rates. It has considerable predictive power, since all this information can be calculated as soon as the depth parameter is fixed by comparison with the energy of a single state. It remains to be seen to what extent it will also be successful for other nuclei. It would be particularly interesting to use the alpha-cluster wavefunctions in analyses of alpha-transfer reactions, and hence obtain information on the alpha-



Comparison between the theoretical and experimental energies of the $K^\pi = 0^+$ and 0^- rotational bands in ^{16}O . The theoretical energies are calculated from an alpha- ^{12}C folded potential with depth parameter $f = 1.425$ fm for the 0^+ band and $\tilde{f} = 1.55$ fm for the 0^- band. The arrow indicates the energy of the $\alpha + ^{12}\text{C}$ threshold in ^{16}O . (Buck, Dover and Vary, 1975).

Table 2 Comparison of various calculated values of the alpha-core separation and RMS radii for the ground state band of ^{20}Ne

J^π	ρ	$\rho^{(1)}$	$\rho^{(2)}$	$\rho^{(3)}$	$\langle r^2 \rangle^{1/2}$
0^+	3.67	3.82	3.75	3.86	2.96
2^+	3.68	3.85	3.78	3.84	2.97
4^+	3.58	3.81	3.76	3.76	2.95
6^+	3.44	3.78	3.76	3.67	2.92
8^+	3.06	3.48	3.56	3.54	2.85

Data from Buck, Dover and Vary (1975). All figures in fm.

Table 3 Theoretical and experimental electromagnetic transition rates for the ground state band of ^{20}Ne

Transition	$B(E2)_{\text{exp}}$	$B(E2)_{\text{th}}$	$B(E2)_{\text{SM}}$	$B(E2)_{\text{RM}}$
$2^+ \rightarrow 0^+$	57.3	57.3	52.6	57.6
$4^+ \rightarrow 2^+$	71.0	73.8	64.5	82.4
$6^+ \rightarrow 4^+$	66.0	62.7	53.4	90.8
$8^+ \rightarrow 6^+$	24.0	28.9	32.8	95.6

Data from Buck, Dover and Vary (1975). Units of e^2 fm.

spectroscopic factors and formation probabilities, which could be compared with values obtained in other ways. $\square$

Immunoglobulin surprises

from C. C. F. Blake

THE recent X-ray determinations of the structures of immunoglobulin Fab's and Bence-Jones proteins have enabled the crystallographers involved to correlate the structure with existing data, and at the same time to extract new and unsuspected information. Poljak in a recent review in *Nature* (**256**, 373; 1975) shows how satisfyingly the large body of chemical and immunogenetic data can be brought together in the light of the molecular structure to provide a unified view of the immunoglobulins.

In accord with this unified view Padlan and Davies (*Proc. natn. Acad. Sci. U.S.A.*, **72**, 819; 1975) have elegantly analysed the structural variability in the combining site. They compared the structures of the V_H , V_L and C_L domains of the murine $\alpha_1\kappa$ Fab McPC603 and V_L domain of the kappa-type Bence-Jones protein REI, using an interesting variation of the diagonal plot. In its more usual form the distances R_{ij} between the i th and j th atom in a molecule are plotted on a diagonal matrix against residue number and contoured at constant separation distance. Secondary structural elements produce characteristic forms of the contour surface and the whole plot reduces the three-dimensional structure to a comprehensible two-dimensional form. In the variation proposed by Padlan and Davies, appropriate for comparing similar structures, the quantities $R_{ij}^A - R_{ij}^B$ are plotted against residue numbers; where R_{ij}^A and R_{ij}^B are the corresponding interatomic distances in the two immunoglobulin domains A and B that are being compared. These difference plots show a number of interesting features, including the close structural resemblance between the V and C domains, but undoubtedly the most important is the concentration of structural differences in the hypervariable regions of the V domains. Padlan and Davies make a most telling correlation by plotting the 'average relative displacement' which represents the change in average distance between a particular residue and all the 'framework' residues in the structure from one domain to the other, against residue number. This plot bears a quite remarkable similarity to the plots of sequence variability and demonstrates very clearly that the hypervariability of sequence is paralleled by an equivalent structural variability. Thus the hyper-

variable regions, by causing insertions and deletions that change the size and shape of the combining site and amino acid substitutions that alter its chemical nature, provide the immunoglobulin molecule with an almost unlimited potential for antigen binding and antibody diversity within an otherwise constant molecular framework.

Although it is encouraging to see this model emerge from the X-ray studies, it was of course already implicit from the studies of sequence constancy, variability and hypervariability. By contrast an aspect of immunoglobulin structure that could not have been foreseen is the subject of a paper by Edmundson *et al.* (*Biochemistry*, **14**, 3953; 1975). Now that the X-ray studies have shown a structural homology between the V and C domains, it is clear that all the domains in the γ globulins have been derived by divergence from a single common ancestral gene. Edmundson and his colleagues have addressed themselves to the most important question in this context: what changes have led to the functional differentiation of the constant and variable domains? The X-ray studies have shown that the basic immunoglobulin fold has a four-stranded and a three-stranded β sheet. In the Fab's and Bence-Jones proteins the constant domains pair through the four-stranded sheets with hydrophobic interactions in a solvent-free interface, while in the variable domains the three-chain layers face each other across a solvent-filled channel that forms the combining site. On this basis Edmundson and his colleagues suggest that the V and C domains are rotational allomers and show that 38 pairs of homologous residues in the two domains are related by a rotation of 163° – 167° about an axis that passes through the intrachain disulphide bridges near the centre of each domain. They also argue that the sequence patterns in the β sheet correlate with this model. For example, in the C domain of the Mcg Bence-Jones protein the normally alternating pattern of polar and apolar residues is broken in the four-chain layers by substitution of hydrophobic residues for hydrophilic residues at the sites of interaction in the C_1 – C_2 interface. Similarly in the V domains the pattern is interrupted in the three-chain layers by substitution of aromatic for aliphatic polar residues in positions important for the maintenance of the geometry of the binding site. Edmundson believes that these genetic changes have led to rotational allomerism of the V and C domains such that homologous regions in the two domains perform different functions, and concludes that this unusual event was of critical importance in the evolution of the immunoglobulins. □

Many models for cell growth and development

The 8th Harden Conference—Control Systems in Normal and Malignant Cells—was held at Wye, UK, on September 14–19.

from Robert Shields

THE most striking impression left by the conference is not that there are too few model systems to study growth regulation but too many. Only a few of these are easy to manipulate *in vitro* but unfortunately these are often the least interesting biologically.

Perhaps the most exciting system which is just beginning to be amenable to *in vitro* manipulation is provided by teratocarcinomas. For some time it has been claimed that teratocarcinomas contain pluripotent mouse embryo cells (termed embryonal carcinoma) but convincing evidence that this was so has so far been lacking. In an elegant demonstration R. L. Gardiner (Oxford University) showed that chimaeric mice could be obtained when the inner cell mass of mouse blastocyst was replaced by clumps of embryonal carcinoma which had been grown *in vitro*. The animals were chimaeric not only for coat colouring but also for isozyme markers, proving conclusively that embryonal carcinoma cells are truly pluripotent. The availability of large amounts of what is essentially embryonic material opens up a whole range of intriguing avenues for study using methods described by other speakers.

One of the most fascinating embryological problems is that of how position in the early embryo influences subsequent development. The great problem with this kind of study is to mark genetically an early embryo cell in such a way that its progeny can be recognised at later stages. Potentially this might be done by making metabolic mutants of embryonal carcinoma cells *in vitro* and transferring these to the blastocyst. These cells could survive in the developing embryo if they were capable of metabolic cooperation and their descendants could be recognised later in development. Work described by J. D. Pitts (University of Glasgow) suggests that metabolic cooperation takes place in many cell types *in vitro*, even between cells from species as diverse as *Xenopus* and hamster. Experiments with metabolically defective embryonal carcinoma cells might prove that similar events occur *in vivo*.

A promising *in vitro* approach to developmental processes using Friend

erythroleukaemic cells was described by P. Harrison (University of Glasgow). These cells appear to be arrested pro-erythroblasts and can be persuaded to differentiate by addition of DMSO. This provides valuable information on the timing of gene transcription and translation since globin messenger sequences can be detected using complementary DNA probes. By making mutant cells unable to differentiate and fusing these with other cells and looking for complementation it is proving possible to dissect genetically the processes that control haemoglobin production.

The sessions on cell growth control were predictably dominated by the search for the message that transmits signals received at the cell surface to the internal machinery of the cell. The current dogma is that macromolecular mitogens act at the cell membrane and do not enter the cell. As J. Kay (University of Sussex) pointed out, however, at least one macromolecule, ricin toxin, exerts its effect by entering the cell, so even the concept of surface receptors may need re-examination. A further complication is introduced by heterogeneity of mitogens and of cell types. Lymphocyte workers have often congratulated themselves on being able to work with well defined mitogens; however, the talk by F. Melchers (Institute for Immunology, Basel) showed that this is often paid for by using heterogeneous cell types in different stages of development. On the other hand, those working with cultured cells can work with a cloned cell type but have to put up with a heterogeneous mitogen, serum. In spite of this, one of the striking things about the action of different mitogens on many different cells is that the metabolic processes stimulated are very similar; what goes for the fibroblast goes for the lymphocyte. The problem is to sort out which metabolic changes cause and which are associated with cell growth.

Using pig T lymphocytes, M. J. Crumpton (MRC National Institute for Medical Research, Mill Hill) showed a close correlation between phosphatidylinositol (PI) turnover and mitogenic stimulation with a whole series of mitogenic and non-mitogenic lectins, however PI turnover was not increased by phorbol ester which is a potent mitogenic agent, showing that PI turnover is not the primary mitogenic event nor is it a necessary signal for cell proliferation. Furthermore a limited degree of lymphocyte stimulation can be produced by the calcium ionophore which also stimulates PI turnover, showing that PI turnover may be a consequence of increased calcium flux into the cell.

Another early event in the induction of cell proliferation is the stimulation of potassium entry by the Na^+/K^+

ATPase. Using cultured 3T3 cells, E. Rozengurt (Imperial Cancer Research Fund, London), could separate the serum-induced stimulation of potassium transport from the increase in phosphate transport and also from the concomitant fall in cyclic AMP levels. The role of cyclic GMP (if any) in the control of growth is still obscure. Data presented by L. Jimenez de Asua (ICRF, London) showed that different stimuli can raise cyclic GMP in 3T3 cells to the same level and yet have very different degrees of mitogenic activity. This suggests that cyclic GMP is unlikely to be the sole trigger for increased growth.

Not all relationships between the cell's interior and the environment need be conducted with chemical messages, physical interaction is also feasible, possibly through proteins which span the cell membrane. Untransformed cells display a protein of about 250,000 daltons on their surfaces, and this protein disappears when the cell is transformed. By making fluorescent antisera to this surface protein A. Vaheri (Helsinki) was able to show that this protein codistributed with actin-containing filaments which lie under the cell membrane. Furthermore, the actin filaments and surface protein disappeared together when the cell was transformed. These results suggest that the surface protein may be linked directly or indirectly to actin-containing structures in the cytoplasm. □

Waxworks for plate tectonics

from Peter J. Smith

AN oceanic ridge and the transform faults that divide and offset it are approximately orthogonal, irrespective of the overall trend of the ridge with respect to the plates on either side. The reasons for this particular geometry are still not entirely clear, or at least not beyond dispute. Most of the explanations put forward so far have involved a minimum energy criterion; in other words, it is generally assumed that the final ridge-fault configuration is that offering least resistance to plate separation. But this implies that when plates diverge the resistive forces per unit length of ridge crest are much greater than those per unit length of transform fault, an inference which is apparently inconsistent with the observation that the seismic energy released at ridge crests is no greater than that released along transform faults.

In an attempt to resolve this dilemma, Lachenbruch and Thompson (*Earth planet. Sci. Lett.*, **15**, 116; 1972)

suggested that the region in which the seismic energy is released and that chiefly responsible for the resistance to plate motion may not coincide. Specifically, they envisaged that whereas the seismic energy is released at the ridge crest in the crust which is continually breaking, the dominant resistive force is the highly viscous fluid in an intrusion zone below. But as Oldenburg and Brune (*J. geophys. Res.*, **80**, 2575; 1975) now point out, the large resistive forces invoked in this and similar models are a consequence of regarding the lithosphere as a slab of constant thickness and the intrusion zone as a narrow vertical channel beneath the ridge crest—a situation in which the plate thickness is much greater than the width of the intrusive channel. Their own model, by contrast, suggests that this condition may not obtain.

But whereas the Lachenbruch-Thompson model is conceptual, Oldenburg and Brune have constructed a real physical system. They have a tray of melted wax which is cooled until a film of solidified wax has formed between one end of the tray and a moveable paddle. When the paddle is pulled at constant velocity away from the solidified surface in which an initial zone of weakness has been produced, a miniature system of ridge segments and perpendicular transform faults is "easily attainable"—a remarkable imitation of a phenomenon discovered less than 20 years ago, using apparatus which could in principle have been constructed more than 2,000 years ago.

Whether this physical model leads to conclusions that are more or less applicable to the real Earth than those derived from its more theoretical predecessors remains to be seen. In the meantime, however, Oldenburg and Brune believe that it can provide valuable insight into oceanic ridge processes because it can be observed directly on a reasonable time scale, because quantitative measurements may be made and because the properties of the system may be varied and their effects noted. It is a simple matter, for example, to measure the thickness of the solidified surface film of wax at any point in the tray; and 'plate' thickness thus obtained is always found to increase as the square root of distance from the 'ridge'. Clearly this direct result conflicts with the constant thickness assumption built into the Lachenbruch-Thompson and other analyses.

If the wax is to be believed, the idea of high resistive forces at ridge crests may also have to be discarded, for observation shows that the ratio of the thickness of the 'plate' to the width of the intrusion zone can be quite small in the vicinity of the 'ridge crest'.

Moreover, order of magnitude calculations by Oldenburg and Brune suggest that the resistive forces per unit length of 'transform fault' are at least two, and possibly as many as four, orders of magnitude greater than those per unit length of 'spreading ridge'. Because so little is known about the physical and dynamic properties of Earth materials at depth, it is difficult to carry out comparable calculations for the real Earth. Nevertheless, assuming that the top few kilometres of the ridge crest act as a vertical channel and that the region below (defined now by a lithosphere increasing in thickness away from the ridge) is a zone of partial melt with Newtonian viscosity, Oldenburg and Brune estimate that the resistive forces along a transform fault are probably about an order of magnitude greater than the viscous forces acting beneath the few kilometres thick crust at a ridge crest of the same length.

Although such calculations must be treated with reserve, they do suggest that previous estimates of the viscous forces at ridge crests may be too high because of an inappropriate geometry assumed for the intrusion zone. Maintenance of the minimum energy criterion for orthogonal spreading would therefore require a large resistive contribution from the upper few kilometres of the ridge crest, thus restoring the seismic energy release contradiction. The only way out would seem to be to abandon the minimum energy assumption for the Earth, just as observation and calculation show it to be inapplicable to the wax model.

Instead, what seems to be crucial in producing the orthogonal pattern is the particular combination of physical properties involved. The wax model does not always lead to perpendicular ridge-fault spreading; it is necessary to use the 'right' kind of wax, and to adjust the temperature of the wax, the rate of surface cooling and the rate of spreading. The wax experiments show, however, that as far as the material is concerned the critical conditions for formation of the orthogonal pattern may be summarised as a single dimensionless parameter G , the ratio of the shear strength of the solid to the resistive stresses along the transform fault. The pattern may only be maintained as long as $G > 1$. Once this criterion is satisfied, the development of the pattern is then determined by the symmetry of the applied stress field and the ability of the wax to fracture in a brittle manner under the applied stresses. In the Earth, both symmetry and brittle fracture are assured. G is more difficult to assess, but preliminary estimates suggest that it may be as high as 30. □

Insect development

A symposium on Insect Development was held by the Royal Entomological Society in London on September 18–19.

from our *Insect Physiology Correspondent*

THE biennial symposia organised by the Royal Entomological Society of London, which have now been running for fifteen years, are unusual in that their purpose is primarily educational: to provide an opportunity for entomologists to learn in comparatively simple terms, from researchers at the fringes of knowledge, what is going on in a given field of study. The latest symposium was organised by P. A. Lawrence around the topic of the genesis of pattern development at all stages from egg to adult. Of the twelve contributors more than half came from overseas. The meeting, with an attendance not far short of 200, attracted students of growth outside the insect world and they were gratified to learn that insect development was nothing like so mosaic as they appear to have believed.

H. A. Schneiderman (University of California, Irvine) gave a general survey of the wide range of work going on in the experimental analysis of determination in the cortical cytoplasm and in the imaginal disks of *Drosophila*. He did not carry all the audience with him in his distinction between 'specification' for cell commitments which are hereditary and 'determination' for commitments which are non-hereditary. K. Sander (University of Freiburg) provided an introduction to insect embryology with emphasis on the nature of movements in the embryo. Evidence from exceptionally clear time-lapse photography indicates that the centrifugal movements of the nuclei at cleavage are effected by tension generated by fibrils of the nuclear asters extending to the cortex; and that the later invaginations result from tension on the blastoderm by cells in the yolk. Using a somewhat different terminology from Schneiderman, K. Kalthoff (University of Freiburg) spoke of cell determination and differentiation, but of pattern specification and realisation. Recent work by the Freiburg group has tended to support a gradient model of determination in the cortical plasma of *Drosophila*, with some evidence that masked messenger RNA may be the chemical agent. But a generalised interpretation of these observations suggested a temporal progression from a gradient state to a mosaic state, the timing varying in different insects and

being most precocious in the highly evolved forms. This sounded very much like a modernised expression of the Seidel view of things. K. Illmensee (Institute for Cancer Research, Philadelphia) had shown that genetically labelled cleavage nuclei and blastoderm nuclei from all parts of the *Drosophila* egg could contribute to any somatic structures and even to the germ line, and that the cytoplasm of the oosome could induce germ cells elsewhere.

Turning to the imaginal disks of *Drosophila*, W. Gehring (University of Basel) described the results of injection of dispersions of genetically labelled cells into wild type hosts, and their ultimate differentiation. Whereas there was already some determination at the two ends of the germ band, at the imaginal disk stage the cells were biochemically distinct. This was well seen in the aldehyde oxidase activity acquired by the antennal disk as contrasted with the wholly negative eye disk. R. Nöthiger (University of Zurich) illustrated the immense value of clonal analysis in the study of imaginal disks. Mutants induced by X-irradiation, followed by mitotic recombination, at different points in development, can demonstrate the

timing of decision taking, and the stability of the resulting determination. J. R. S. Whittle (University of Sussex) emphasised the caution needed in the use of mutants in morphogenetic studies where, for example, biochemical abnormalities could provoke pleiotropic defects in growth. P. A. Lawrence (University of Cambridge) described the important results of Garcia-Bellido and his colleagues in Madrid in the definition of 'compartments' of the integument derived from the lineage of single cells or groups of cells and visualised by X-ray-induced mitotic recombination. Even when using such mutant cells in a polyclone which far outgrows the surrounding cells, the mutant cells never intrude beyond the compartment boundary—such as exists in the longitudinal mid-line of the *Drosophila* wing or at the intersegmental boundary of the *Oncopeltus* abdomen. Recent evidence obtained in Cambridge has made clear that this remarkable restriction depends on a failure of mutual intercellular adhesion with cells outside the compartment, a property carried by special genes. The results of further studies of the significance of this new genetic unit of development will be awaited with great interest. □

Plant collections and conservation

Recommendations for the preservation of endangered plants have come from an international conference held at the Royal Botanic Gardens, Kew on September 2–6. Participants from 28 countries, meeting to consider the function of living plant collections in conservation and related research and public education, produced some resolutions calling for early action.

They include the following:

- A worldwide network of nature reserves and gardens oriented towards conservation should be established, and institutions in temperate countries should provide technical aid and personnel through the auspices of the International Union for Conservation of Nature and Natural Resources.
- Special attention should be given to the conservation of threatened floras of islands and areas with Mediterranean and similar climates, where many endemic species are endangered by human activities.
- Institutions with plant collections are recommended to give

priority to the local flora, so as to benefit from available specialist knowledge and to reduce the need for simulated climatic conditions. In this way institutions will be best able to advise and educate specialists and the public about the conservation of indigenous species.

- All governments are urged to ratify the Convention on International Trade in Endangered Species of Wild Fauna and Flora as soon as possible.
- Wherever possible, all living plant collections grown for conservation purposes should also be stored in the form of seeds.
- The propagation of rare and endangered species should be actively pursued by botanic gardens and other bodies maintaining living plant collections, and, when necessary, they should be supported by conservation or other appropriate organisations. Special attention should be given to economic plants and their wild relatives and to plants which may have commercial value.

review article

Letters from Einstein to de Sitter on the nature of the Universe

Carla Kahn & Franz Kahn*

The correspondence between Einstein and de Sitter during 1916 and 1918, recently discovered in the archives of the Sterrewacht at Leiden provides a fascinating glimpse of the thinking of the two principal progenitors of modern cosmology.

In August 1974, when the Sterrewacht in Leiden moved from the buildings which it had occupied since 1861, its archives moved with it. During the time of the removal Professor van de Hulst received a letter from Princeton. Miss Helen Dukas, Einstein's former secretary, was going through Einstein's papers, and had found some letters from de Sitter. de Sitter, who was one of the greatest directors of the Sterrewacht, was also one of the best astronomers of his time. It was thought that there might be corresponding letters from Einstein in the archives of the Sterrewacht.

In the chaos of the move from the old Observatory it was not possible to look for the Einstein correspondence immediately. It was not until we, a visiting professor of astrophysics and his wife, arrived at the Sterrewacht at about the time when the move was over, that a serious search was begun. It was suggested that one of us (C.V.K.) might index the archives, partly with a view to the possibility of finding the letters from Einstein.

Very little had been done with the papers in the past 25 years, and there was no way of finding interesting material, except by searching through all the storage boxes. In fact it was only at the thirtieth box that anything appeared which could be connected with the Einstein letters, but when they did turn up there were seventeen items of correspondence. Some were postcards and some longer letters. All but one were written by hand, and dated between 1916 and 1918. Some had been sent at intervals of about a week, and even so had obviously left time for a reply to be dispatched in between. The postal system seems to have been rather better than it is now, even though Germany was at war.

Most of the letters contained discussions of the theory of general relativity. An important purpose of Einstein's contact with de Sitter was to get this work known in England. In 1915 Einstein's first paper on the subject had appeared in Germany. But there was no communication between Germany and England at the time, and so de Sitter eventually published two long articles on relativity in the *Monthly Notices of the Royal Astronomical Society*. In preparing them he must have been stimulated to think about the subject himself and to make his own contributions. In his letters and postcards Einstein throws an interesting light on his own reactions to de Sitter's ideas.

At that time Einstein included interactions of two kinds in his theory. The first was gravitational: each mass causes a distortion of the space around itself and so affects the motion of other masses. A significant difference between Newton's laws of

gravitation and those of Einstein would only occur when very large differences of gravitational potential were involved, so that a particle moving in such regions would acquire a speed comparable to that of light. Such large potentials are likely to occur only in astronomical situations, which explains the current interest of relativists in black holes. But sixty years ago no one believed in such objects, and they are still somewhat controversial. So Einstein had to turn to the large scale properties of the Universe to find a promising application for his theory. In any case he seems to have been attracted by the age-old problem of whether space is infinite or finite, either of which seems hard to accept. To make a model of the Universe in equilibrium he needed another kind of interaction, in addition to gravitation, for with gravity alone he knew that there could be no static configurations. The general theory of relativity can be formulated so as to allow for the existence of another interaction, the cosmical repulsion. This was described in terms of the so-called Λ term, but the exact value of Λ was not predicted, and could well have been zero. (Nowadays most

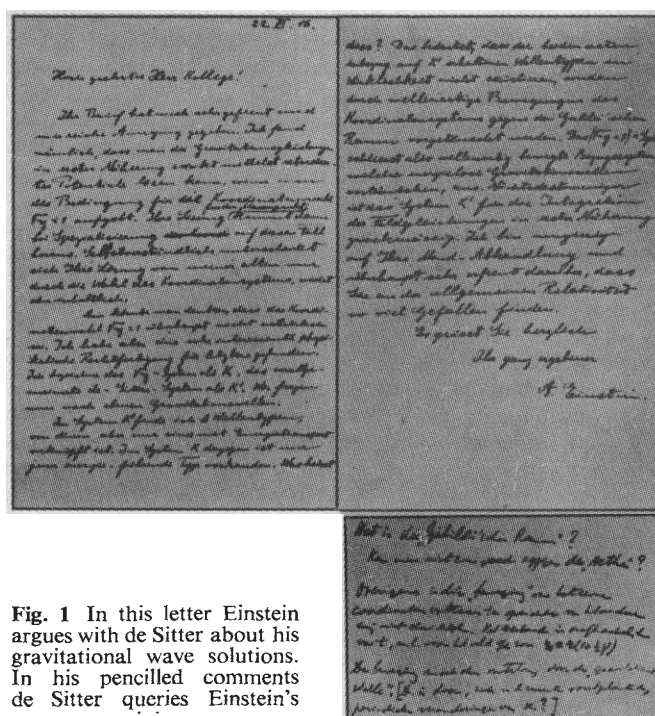


Fig. 1 In this letter Einstein argues with de Sitter about his gravitational wave solutions. In his pencilled comments de Sitter queries Einstein's opinions.

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This article was first published in Dutch in *Natuuren Techniek*, May 1975.

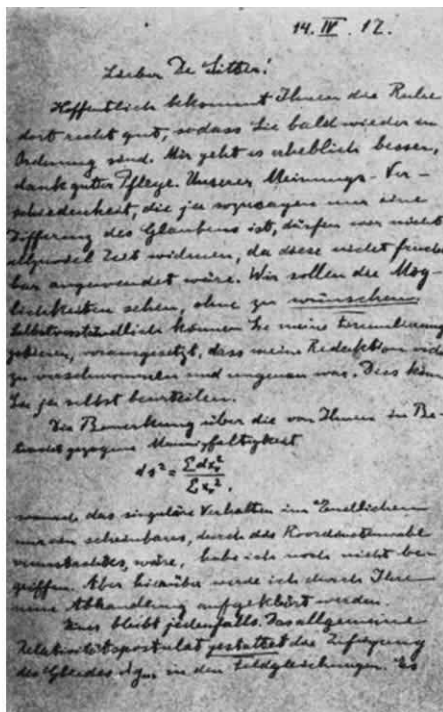


Fig. 2 Einstein expresses the view that one day our understanding of the system of stars will be good enough to determine whether Λ vanishes or not. He concludes "Conviction is a good mainspring, but a bad regulator".

relativists believe, as Einstein did eventually, that Λ is in fact zero.) But with a Λ term it was possible to balance the gravitational self attraction of the matter in the Universe, provided Λ was equal to $(4\pi/3)G\bar{\rho}$, where $\bar{\rho}$ is the mean mass density in the Universe. Naturally one has first to find out what $\bar{\rho}$ is; we shall return to this point later.

The earliest letter in the archives (dated June 22, 1916) deals with the problem of gravitational waves. de Sitter had found that there were three kinds of wave: but Einstein commented that only one kind transported energy. "What does this mean?", he asks. "This signifies that the two former types of wave . . . do not exist in reality", and he goes on to say that the waves disappear when he uses a different coordinate system, which he calls "galilean space", to express his physics.

de Sitter was not happy about this idea. In a pencilled comment he asks: "What is 'galilean space'? Can one not equally say 'the ether'?" Later he concludes that the type of motion he discovered must arise through what he called a gravitational wave.

Two more letters from Einstein continue the argument, but really take it no further. Who was right? If a physicist nowadays comes across a wave-like disturbance without energy, then he immediately suspects that the system carrying the wave is unstable. Years later Lemaître in fact constructed a model universe (with a Λ term) which begins in equilibrium but moves away from it because the equilibrium is unstable. It looks as though de Sitter saw a glimpse of this—and perhaps more—when he was thinking about gravitational waves. But he could not convince Einstein.

There then follow a letter and a card, written in November 1916 and January 1917, respectively. In the letter Einstein speculates at length on the problem of Mach's principle; does the inertia of a given mass depend on the other masses in the Universe, and if so how? This kind of question has been much discussed in the past 20 years or so, but de Sitter seems not to have been greatly interested in it. At the end of his letter Einstein says: "I do not demand in any way that you should share my curiosity". The subject does not occur again in the correspondence.

The postcard is not about scientific matters at all. "It is good of you" Einstein writes "that you are throwing a bridge over this abyss of misunderstanding. With this card you will receive the reprint you asked for, plus some others, for the colleague.

When peace returns I shall write to him . . .". The colleague was presumably Eddington and the abyss the war; Einstein sensibly does not mention him by name in case the German censor read this card.

The following post card was sent on February 2 of that year. Again there is not much science, but rather plaintively Einstein remarks: "There has been no progress with the possibility for a reappointment P., and this looks suspicious to me. There must be some intrigue at work. Naturally I shall hear nothing about it before it is too late".

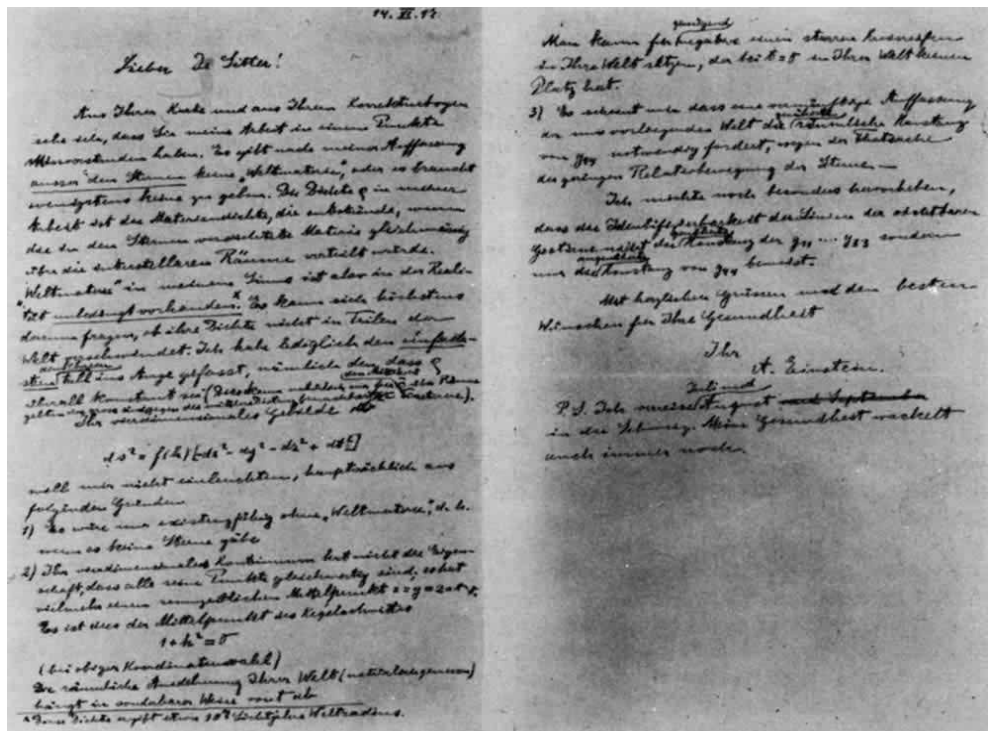
He was right, for in his next letter (March 12) Einstein writes " . . . it is dreadful that they elected M. rather than K. to Potsdam, in spite of the proposal by the Academy . . . It is not clear which powers are responsible. One suspects Seliger.

"Now to our business!" The business was to construct a model for the Universe, and to tie it to some observations. Einstein's starting point was the mass density in space, as determined from star counts. He took the value $\bar{\rho} = 10^{-22} \text{ g cm}^{-3}$; from this it follows by fairly general arguments that the length scale of the Universe must be about $c/\sqrt{(4\pi G\bar{\rho})}$ if $\bar{\rho}$ is representative of all space. Einstein derives a radius $R = 10^7$ light years for the Universe, "whereas", he says, "we see to a distance of about 10^4 light years". Of course we know now that the numbers were bad estimates. The density $\bar{\rho}$ would have referred to the space in the disk of our Galaxy (and even so $10^{-22} \text{ g cm}^{-3}$ is about thirty times larger than the value astronomers have determined now). The density averaged over the Universe is many orders of magnitude smaller, and our present value for the radius of the Universe consequently much larger. Finally the distance "to which we see", at least in the plane of the Galaxy, is restricted by the obscuration due to interstellar dust, and has little to do with the Universe at large.

Einstein was uneasy about the results of his investigation. "I compare space to a cloth . . . one can observe a certain portion . . . we speculate how to extrapolate the cloth, what holds its tangential tension in equilibrium . . . whether it is infinitely extended, or finite and closed. Heine has given an answer in a poem, 'and an idiot expects an answer'". And then he looks forward to his next meeting with de Sitter in Leiden. Now, almost 60 years later, we astronomical "idiots" are still waiting for the answer.

A few days later (March 24) Einstein wrote again. de Sitter had developed a model for the Universe, but Einstein did not

Fig. 3 Einstein makes various critical remarks concerning de Sitter's model of the Universe. His third point is that "a sensible conception of the Universe before us necessarily requires the approximate constancy of g_{44} in space, owing to the small relative motions of the stars". Some years later Hubble discovered the expansion of the Universe.



entirely approve. In particular he thought that he noticed that the Universe contained a singularity (a place where space is so distorted that its geometrical properties break down), and that this happened only a finite interval away, equal to

$$\int ds = \int_0^{1/\sqrt{\mu c}} \frac{cdt}{1 - \mu c^2 t^2} = \frac{1}{\sqrt{\mu}} \frac{\pi}{2} \text{ (finite)}$$

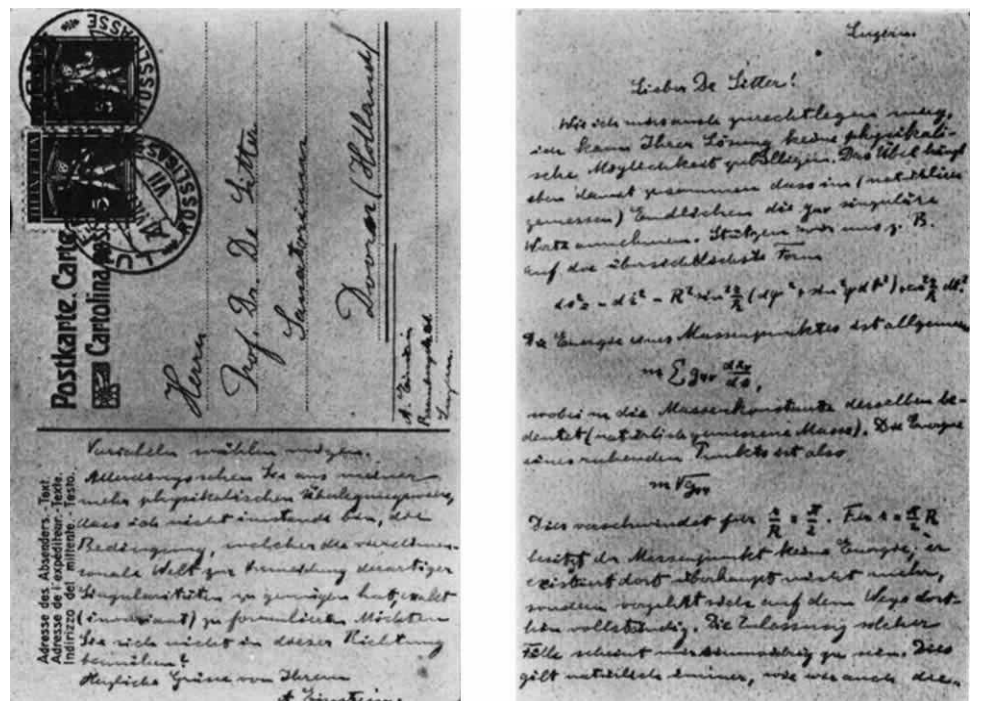
de Sitter crossed out the result in pencil, and wrote "see postcard from Kluyver" (who was his assistant). What his assistant had pointed out was that Einstein occasionally made elementary mistakes in calculus. But he tried to draw far-reaching conclusions. "The surface with singular properties is therefore in physical finite space. Therefore it seems to me that your

solution corresponds to no physical possibility." In this case there is actually no singularity in physical space-time. But one may ask why there should not be a singularity within finite space-time. We willingly accept the idea now. The subject is raised again by Einstein in a letter dated June 14, 1917, in a discussion about the model of the Universe, which is now known by de Sitter's name.

"Your metric does not make sense to me". (A metric is a set of coefficients which specify the geometrical properties of space.) "It could only apply without 'world material', that is stars". But the metric would apply with good approximation to a Universe with a low material density $\bar{\rho}$. Present-day observations are quite compatible with such a low value of $\bar{\rho}$.

Later Einstein says "The spatial extent of your Universe depends on t (time) in a peculiar manner. For sufficiently early

Fig. 4 Einstein objects to the appearance of a singularity in a world model by de Sitter. "To admit such cases seems senseless to me", he writes.



times one can put a rigid circular hoop into your Universe which has no place in it at time $t = 0$ ". The 'Big Bang' evidently did not appeal to him.

But he accepts it in a letter one week later (June 22) in which he gives a geometrical argument to explain why the instant of the Big Bang is special. "This point is thus *de facto* preferred . . . Naturally this does not constitute a disproof, but the circumstance irritates me".

There are several further communications from Einstein after this letter, but they are concerned more with details than with principles.

There are also less scientific parts of the letters. de Sitter and Einstein seem to vie with each other in describing their ill-health; in fact most of the letters are addressed to de Sitter at a sanatorium. Together with this correspondence there were some letters from Eddington, who was Secretary of the Royal Astronomical Society at the time. They concern the papers which de Sitter had written on general relativity. The RAS eventually published them, but at first Eddington's letters were somewhat cool. You understand, he wrote, that every paper published by the RAS has to be sent to a referee. (He would of course have been quite qualified to referee them himself.) We are having trouble with the printers, the paper is longer than we normally print and so on. But in the end the RAS accepted de Sitter's contribution.

Another story which is intriguing has to do with the removal of the Radcliffe Observatory from Oxford to South Africa. It had been set up by John Radcliffe in the eighteenth century, and hardly any student had used it for the past hundred years. (It was Dr Knox Shaw, the Radcliffe observer, who originally proposed that his observatory be moved away from Oxford. In one of his letters to de Sitter he remarks that the students

had made practically no use of his observatory for a very long time. Yet one of Einstein's chief arguments against the removal was that one could not justify the loss of amenities, which the University of Oxford would suffer as a consequence.) Oxford is not blessed with the kind of weather that facilitates observing. A number of influential astronomers felt that it would be useful to move the observatory to Johannesburg, but there was some opposition, led by Lindemann, who was at that time an influential scientist in Oxford. He had enlisted the support of Einstein, while de Sitter was a supporter of the astronomers. Because the observatory was administered by a Trust Fund, the matter had to be decided in the High Court.

de Sitter wrote to Einstein to say that he had seen a letter over Einstein's signature, and that he [de Sitter] could not believe that Einstein knew what he was signing. Einstein wrote back rather crossly that of course he knew what he was signing. de Sitter then sent an affidavit to the proposers of the move in which he said that, though Einstein was a very great scientist, his knowledge of astronomy was minimal and his advice in this matter should be ignored. Counsel for the proposers felt that such a letter would not be helpful to their cause, and de Sitter was asked to remove the reference to Einstein from his affidavit.

The last letter from Einstein was written in 1933. He thanked de Sitter for his offer of help, but said that he was managing "to survive with his own (family)", and was even able to help others "over the water". He did not expect to be able to save very much from Germany, as proceedings had been started against him for high treason.

Soon after this Einstein moved to Princeton, where he lived and worked until his death in 1955. de Sitter's health failed, and he died in 1934.

articles

An accelerating Universe

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New data on the Hubble diagram, combined with constraints on the density of the Universe and the ages of galaxies, suggest that the most plausible cosmological models have a positive cosmological constant, are closed, too dense to make deuterium in the big bang, and will expand for ever. Possible errors in the supporting arguments are discussed.

"If then, Socrates, in many respects concerning many things—the gods and the generation of the Universe—we prove unable to render an account at all points entirely consistent with itself and exact, you must not be surprised. If we can furnish accounts no less likely than any other, we must be content."

Plato, *Timaeus*

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THE cosmological constant has been invoked on several occasions to correct some seemingly real difficulty with the cosmological predictions of standard general relativity^{1,2}. The most notable of these were its initial use by Einstein to produce static universes, the models by Lemaitre designed to explain how the Solar System could be older than the then accepted value of the Hubble time, and most recently by Petrosian *et al.*³ in an attempt to explain the concentration in QSO number counts near $z = 2.0$. In each of these cases either the rationalisation for the introduction of non-zero Λ has disappeared with better data or understanding, or the application has been unsuccessful in its intended purpose.

We seem to be in the situation once again where the data may call for Λ to be dusted off and inserted in the field equations. The rationale now is the almost-zero formal value for the deceleration parameter obtained by Gunn and Oke⁴ which, when reduced by any reasonable evolutionary correction, yields negative values for q_0 much larger than the formal errors.

Such negative values correspond to accelerated expansion and require a net repulsive force. Within the framework of general relativity, a positive Λ is the only way to obtain such a force. (It is perhaps worth noting that relativistic theories of gravity exist which are consistent with Solar System experiments which do not satisfy Birkhoff's theorem and in which accelerated cosmological models arise naturally (C. Caves, unpublished).)

The physical situation regarding Λ is unclear. Most relativists find it repulsive in principle rather than by observation. If it is regarded as a fundamental constant of the classical theory, it does indeed reduce the lustre of an otherwise beautiful framework. Zel'dovich⁸, however, has shown that a Lorentz-invariant stress tensor may arise quite naturally out of quantum fluctuations *in vacuo*, and if so, its form must be $\Lambda g_{\mu\nu}$. If the cosmological term is to be regarded as a piece of the stress tensor rather than a part of the theory, it would seem to be more acceptable.

In any case, comparison of the classical Hubble diagram with density estimates based on 'local' determinations seems the best way of determining the existence of a non-zero Λ . We shall see, in fact, that the Hubble diagram alone may dictate that Λ be non-zero.

The data do not demand this conclusion, and there is the possibility that systematic errors remain, but the suggestion is strong enough that we thought it worthwhile to investigate the models in the light of other relevant observations, in the spirit of the recent work of Gott *et al.*⁶ for models with $\Lambda = 0$.

Properties of Friedman models with $\Lambda \neq 0$

Three parameters specify a model completely. A set usefully related to observables is the density parameter

$$\Omega = 8\pi G\rho_0/3H_0^2$$

(where G is the gravitational constant, ρ_0 is the present density and H_0 is the Hubble constant), the deceleration parameter q_0 ($= -\ddot{R}_0 R_0/\dot{R}_0^2$, where R_0 is the present expansion scale factor), and H_0 . An alternative set that is related to the nature of the function $R(t)$ is the ratio Λ/Λ_c (where Λ_c is the critical value, $\Lambda_c^{-1/2} = 4\pi G\rho R^3/c^2$), which remains constant during evolution of the Universe—and a dimensionless scale factor to specify the present epoch, such as $kR^2\Lambda_c$ and a scale such as H_0 . (Relationships among these quantities are given in refs 3 and 7.) We note now that $\Lambda/3H_0^2 = (\Omega/2) - q_0$, so if $q_0 < 0$, Λ must be > 0 .

Figure 1 depicts the model types in the $(q_0, \log \Omega)$ plane, with small inserts showing schematically the function $R(t)$. Lemaître models, with an extended quasistatic period, occur for Λ only slightly greater than Λ_c . Note that space is hyperbolic, flat, or spherical according to whether the quantity $(3\Omega/2) - q_0 - 1$ is negative, zero, or positive: but if $\Lambda > 0$, closure no longer means necessarily that the Universe will stop expanding, whereas for all models with $q_0 < 0$, the expansion continues indefinitely.

Constraints

To establish constraints on possible model parameters, we need a third dimension (H_0) in Fig. 1. In Fig. 2 this is represented partially by separate $(q_0, \log \Omega)$ diagrams for two values of H_0 , using an expanded scale in the area that will include any viable models (the parameter space studied by Gott *et al.* is represented by the lines $\Lambda = 0$).

Maximum redshift. If $q_0 < -1$, then models with a maximum redshift, z_m (that is a minimum R) occur if $\Omega < \Omega_c(q_0)$, the value of Ω on the line $\Lambda = \Lambda_c$. The value of z_m at a given q_0 is then less than a critical value $z_c(q_0)$, shown in several places in Fig. 1. The existence of a QSO at $z = 3.53$ immediately shows that if $q_0 < -1.13$, models below the critical line are ruled out. More seriously, thermalisation of the background radiation requires a redshift of at least 100. Models with such a

large maximum redshift have $\Omega < 2 \times 10^{-6}$, which is impossible, so we can eliminate at once the whole parameter range $q_0 < -1$, $\Lambda < \Lambda_c$. This constraint is not shown in Fig. 2, since it will be implied by a more stringent limit later.

Ages of galaxies. A lower limit to the age of the Universe (t_0) is given by limits for the ages of stars and elements in the Galaxy, $\gtrsim 8 \times 10^9$ yr (ref. 6). This rules out models with $H_0 t_0 < 0.40$ and 0.64 for $H_0 = 50$ and $80 \text{ km s}^{-1} \text{ Mpc}^{-1}$ respectively, which lie above and to the right of the areas shown in Fig. 2 (compare the $H_0 t_0$ contours in Fig. 1), so will be eliminated by stronger constraints.

To set an upper limit is more complicated. A reasonable upper limit to the ages of globular clusters is 16×10^9 yr (compare the references cited in ref. 6); nucleochronometers cannot provide a model-independent upper age limit for the Galaxy⁸.

Evolutionary models for giant elliptical galaxies predict that their colours would be redder than is observed for ages greater than 16×10^9 yr, if their composition is solar (B. M. Tinsley and J. E. Gunn, see ref. 20). Their spectra indicate an overabundance of metals, if anything, relative to the Sun⁹⁻¹³, so we take as a safe upper limit 18×10^9 yr, corresponding to an underabundance by a factor of 2. Though it seems natural in most theoretical scenarios to form galaxies at a time roughly equal to the dynamical time of the galaxy (a few times 10^8 yr), galaxies can form later, given enough dissipation to enable them to evolve to their present condensed states. The perturbation equations (see, for example, ref. 14) require that galaxy formation occurs before the time when Ω becomes small. This is a significant constraint for small H_0 ; the locus of those models for which $\Omega = \frac{1}{2} 18 \times 10^9$ yr ago is shown in Fig. 2a. Models near this limit in the $k = +1$ region have relatively small redshifts at the latest allowable epoch of galaxy formation and are thus afflicted with very large evolutionary corrections (see below) and are inconsistent with the limits on the blue-visual cosmic light^{15,16} unless the radiation from

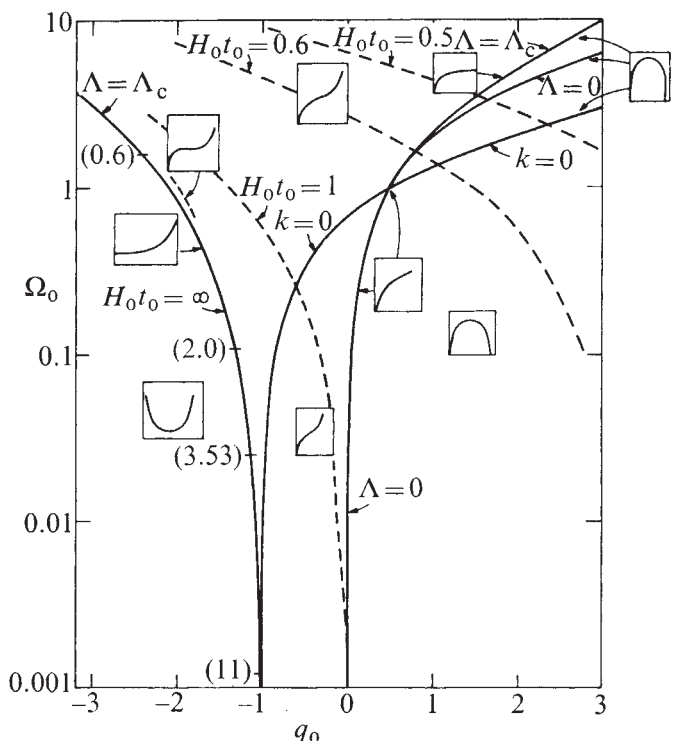


Fig. 1 Friedman models in the $(q_0, \log \Omega)$ plane. Solid curves show the models with $\Lambda = 0$, $\Lambda = \Lambda_c$, $k = 0$ as indicated. Dashed lines give dimensionless ages, $H_0 t_0$ (which are infinite at and below the critical curve for $q_0 < -1$). Small inserts show the expansion function $R(t)$ schematically. Numbers in brackets on the critical curve show the limiting redshift for asymptotic (Eddington) models on this curve, which is greater than the maximum redshift in models below or to the left of that point.

young galaxies is strongly absorbed. Galaxies can form shortly after decoupling at large redshifts only if $t_0 \lesssim 18 \times 10^9$ yr; this line is shown in Fig. 2a and b. For $H_0 = 30 \text{ km s}^{-1}$, all models with $\Lambda \geq 0$, $q_0 < 1$ are older than this.

Density. A lower limit to Ω is the contribution, Ω^* , of matter associated with galaxies. Gott *et al.* estimate $0.04 \lesssim \Omega^* \lesssim 0.08$, and we consider that an interval no greater than $0.02 \lesssim \Omega^* \lesssim 0.3$ is consistent with all the evidence discussed. The dynamical effects of Λ will invalidate an estimate of Ω^* in a region with $\rho < |\Lambda|/4\pi G$, that is, with density contrast $\rho/\rho_0 < 2|\Lambda|/$

$3\Omega H_0^2 = |\Omega - 2q_0|/\Omega$. All of the models that will be allowed in Fig. 2 have $(|\Omega - 2q_0|)/\Omega \lesssim 100$ so they are consistent *a posteriori* with the use of Ω^* from the dynamics of bound clusters. It may not, however, be valid to derive an upper bound on Ω^* from the uniformity of the Hubble flow in some models.

Figure 2 shows the lower bound $\Omega > \Omega^* > 0.02$, which is independent of H_0 .

An upper limit to Ω is not so safe because matter could be hiding outside galaxies or clusters⁶. The requirements on smoothness are less severe if $|\Lambda|/4\pi G$ is significant compared with the mean density, but the least contrived situation is that $\Omega \sim \Omega^* < 0.3$. This value is shown as an upper bound in Fig. 2.

A more stringent upper limit is obtained if we believe that deuterium is produced in the big bang. Studies of nucleosynthesis in supernova shocks, subsequent to those cited by Gott *et al.*, have not provided a clearcut answer as to whether supernovae could be the source of galactic deuterium^{17,18}.

If production in a 'canonical' big bang is required, then the best estimate⁶ is $\rho_0 < 4 \times 10^{-32} \text{ g cm}^{-3}$, which is indicated as a possible upper limit to Ω in Fig. 2.

Quasars. In Lemaitre models, a peak in the redshift distribution of extragalactic objects is predicted at the inflection redshift z_i , at which q changes sign from positive to negative; its value is given by $(1+z_i)^3 = (\Omega - 2q_0)/\Omega$. From the absence of a significant peak in QSO counts at $z < 2$, and from the calculations of Petrosian and coworkers^{1,3,19} we believe that models with $1 < \Lambda/\Lambda_c < 1.1$ ($k = +1$) and $z_i < 2$ can be ruled out. In fact, we believe that all ($k = +1$) models for which the radial coordinate reaches π at a redshift $z\pi < 3$ can be ruled out. These models have a range of redshift around $z\pi$ for which objects are very bright, and it is unlikely that bright galaxies and quasars from such redshift ranges would have escaped detection. This limit is a bit more stringent than the one applied to the counts, and the forbidden region is indicated by the solid black area at the left of Fig. 2a and b.

Hubble diagram. The interpretation of the Hubble diagram is quite complex, and its understanding is contingent on understanding the evolution of elliptical galaxies. Ignoring evolution, Gunn and Oke⁴ get an apparent value $q_{0a} = -0.15 \pm 0.57$ (1σ) for their favoured statistical sample, from a set of galaxies most of whose weight for this problem comes from redshifts in the vicinity of 0.3.

This is consistent with the classical steady-state value $q_0 = -1$, within the errors, in which case no evolutionary correction can be applied. The steady-state model seems, however, to be very unlikely on other grounds, such as production of the microwave background radiation.

The evolutionary correction from population syntheses of ellipticals is (ref. 4 and B.M.T. and J.E.G., unpublished).

$$\Delta m(z) \simeq -(1.3 - 0.3x) \ln \left\{ \frac{t_0 - t(z)}{t_{\text{gal}}} \right\}$$

where t_0 is the present age of the Universe, $t(z)$ is the age at z and t_{gal} is the present age of the Galaxy; x is the slope of the initial mass function. The first order correction to q_0 from this magnitude change is

$$EC = q_{0a} - q_0 = \frac{2.5}{\ln 10} \frac{2}{2-\alpha} \frac{1.3-0.3x}{H_0 t_{\text{gal}}}$$

where $\alpha \sim 0.7$ is the slope of the flux-diameter relationship for ellipticals⁴. For reasonable values of the parameters, EC is always positive.

Results on red and infrared line indices demand that $x \leq 1$ if the initial mass function can be represented by a power law (B. M. Tinsley and J. E. Gunn, unpublished). We have thus taken $x = 1$, since this must underestimate $|EC|$, and we have

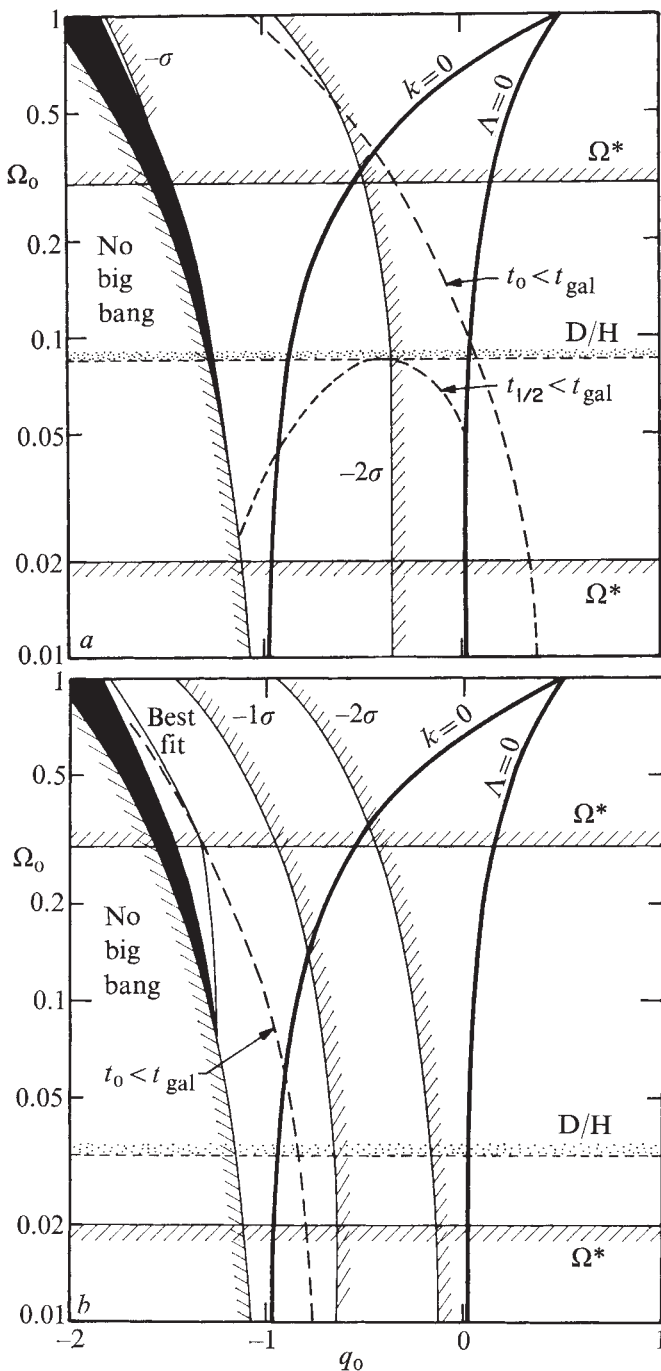


Fig. 2 Constraints in the $(q_0, \log \Omega)$ diagram for: a, $H_0 = 50$ and b, $H_0 = 80 \text{ km s}^{-1} \text{ Mpc}^{-1}$. Heavy lines correspond to $\Lambda = 0$, $k = 0$ as in Fig. 1. The two boundaries labelled Ω^* are limits on the dynamically detectable density, and the density limit D/H is an upper bound if deuterium is cosmological. The solid black area is for models with $0 < (\Lambda/\Lambda_c) - 1 \ll 1$ which are ruled out by the absence of focusing effects; all models with $\Lambda < \Lambda_c$ (no big bang) are ruled out. Constraints based on the ages of galaxies (t_{gal}) are discussed in the text, as are those based on the Hubble diagram and labelled best fit, -1σ , and -2σ .

likewise taken the largest possible number for t_{gal} ; that is, t_0 or 18×10^9 yr, whichever is smaller. We then constructed Hubble diagrams for a set of models in the Ω, q_0 region of interest ($\Lambda \geq 0$, $0.01 < \Omega < 1$, $\Lambda > \Lambda_{\text{crit}}$ for $k = +1$). The Malmquist correction was applied as outlined previously⁴ for a dispersion in absolute magnitude $\sigma = 0.4$. The standard deviation in q_{0a} was then converted into a magnitude difference at $z = 0.3$. This simplification enables the goodness of fit of any model to be checked approximately by calculating its predicted magnitude at $z = 0.3$ and comparing this with the value given by q_{0a} with no EC and the 1σ and 2σ limits given by the formal errors on q_{0a} . The approximation should be very good, since the relevant data are strongly concentrated at about this redshift, and the comparison is, to first order, independent of redshift. The results, in the form of best fit lines and contours at 1σ and 2σ are plotted on Fig. 2a and b. All models are ruled out at the 2σ level if H_0 is as small as 30.

For $H_0 = 50$ (Fig. 2a), there is almost no model which agrees within 1σ , none which satisfies the Ω^* constraint, and certainly none which makes deuterium. At the 2σ level, a wide variety of models is possible, including models of both curvatures. None of these models which satisfy the Ω^* constraint can make galaxies early; and all must have positive Λ .

For $H_0 = 80$, the ages are much smaller, and the age constraint on the galaxies easier to satisfy. A larger collection of models can make galaxies early, and thus the evolutionary corrections can be smaller. There is a small portion of the best fit line in the allowable region on this diagram, but none of these models can make deuterium, and all are close enough to $\Lambda = \Lambda_{\text{crit}}$ that they have peculiar Hubble diagrams at large redshifts ($z \sim 2-3$), and may be ruled out on that basis in the future. All are closed. Models at the -1σ and -2σ levels can make galaxies early, can make deuterium, and can be either open or closed. Again all require that $\Lambda > 0$, but the -2σ contour approaches the $\Lambda = 0$ locus very closely for small Ω . It is perhaps worth noting that if the Ω^* level is put in at the 'best' value of 0.06 found by Gott *et al.*, no models with H_0 this large can make deuterium. All of these remarks about deuterium of course refer to the 'standard big bang' nucleosynthesis models and can be relaxed somewhat if one considers various complications (see ref. 6).

Summary of constraints. Thus it would seem that the data suggest (1) that the universe is closed, (2) that the density is fairly high ($\Omega \gtrsim 0.1$), (3) that $\Lambda > 0$, and in fact, that Λ is within a factor 2 of Λ_{crit} , (4) that H_0 is at least about $80 \text{ km s}^{-1} \text{ Mpc}^{-1}$, and (5) that deuterium is not primordial. We are, of course, not compelled to believe all of these conclusions, since within 2σ of the best fit are a large number of other possibilities, but no models with $\Lambda = 0$; this state of affairs is independent of H_0 , but can become more stringent if H_0 is small enough that galaxies are made late. All of the even remotely allowable models expand forever. The $q_0 = 1/2$, $\Omega_0 = 1$ model which is the 'closest' boundary point of the set of $\Lambda = 0$ models in the $q-\Omega$ diagram in which the models undergo collapse in the future is 4σ away from the observations.

Conclusion

The first reaction to all this is that something must be terribly wrong, and we conclude therefore with some of the possible pitfalls.

Systematic effects in the photometry. Since the low and high redshift samples in Gunn and Oke's analysis⁴ had to be treated differently due to technical difficulties, there is a possibility that the two sets are not corrected properly to the same system. It seems highly unlikely that the error is as large as 0.05 mag, however, which corresponds to an error in q_0 of less than 0.25. An increase in the measured q_0 by this amount would make the fitting problem less extreme but would not substantially change the situation presented here.

Intergalactic obscuration. An absorption of 0.2 mag at $z = 0.3$ will decrease the measured q_0 by 1. This requires a gas density

corresponding to $\Omega \sim 0.03$ if there is the same dust-gas ratio as the interstellar medium in the solar neighbourhood. The presence of so much dust seems *a priori* exceedingly unlikely, but it may be possible to create scenarios in which this much nuclear processing occurs and is followed by ejection of grains into intergalactic space.

Galactic evolution. The evolutionary corrections are of course crucial to the interpretation given here, and one may reasonably ask how uncertain they are. In our opinion there are two major sources of uncertainty. First, the power-law approximation to the main-sequence mass function may be seriously misleading. Until careful and accurate evolutionary effects on galactic spectra can be observed, this will be difficult to check. Second, there may be serious troubles with stellar evolution theory (we clearly do not understand the Sun) but empirical checks with galactic clusters and the old disk (B. M. Tinsley and J. E. Gunn, unpublished) make large errors here a bit unlikely. The constraints we adopted on the age of galaxies, however, are critically dependent on the synthetic colours and could possibly be in error. Since our conclusions about the Hubble constant are affected strongly by this constraint, they should be regarded with due caution.

Dynamical evolution. It is possible that the central galaxies in clusters could undergo accretion of other cluster members through dynamical friction effects on a time scale which is sufficiently short to affect significantly the evolution of the total light. Preliminary estimates indicate that the effect is probably not important, but there are a great many uncertainties, including the question of how much of the cluster mass resides in the galaxies themselves, and whether clusters typically have a cold central subsystem from which accretion could occur rather efficiently. It seems futile to try to estimate the effect more accurately until at least these questions are answered.

Density limits. The constraint on Ω^* is weakened somewhat by the possible existence of a non-zero Λ , since the local supercluster flow uniformity is no longer an effective argument, but the other arguments seem still quite persuasive. It is of course not clear what the status of the primaeval deuterium production constraint is, nor will it be until one can reasonably rule out subsequent production.

It is clear that if H_0 is small, the age constraint on galaxies is correct, and q_0 is large and negative, then deuterium probably cannot be primordial, Ω cannot be small, and one cannot escape the conclusion that Λ is non-zero and positive. The incorrect piece(s) of the puzzle are well hidden, and the situation is, as is usual in observational cosmology, that one awaits new and better data.

This work was supported in part by grants from the National Science Foundation.

Note added in proof: More detailed calculations by J. P. Ostriker and S. D. Tremaine (*Astrophys. Lett.*, in the press) and S. D. M. White (unpublished) show that the effect mentioned in the paragraph "Dynamical evolution" may be more important.

Received July 10; accepted August 21, 1975.

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Identification of two copies of the gene for the elongation factor EF-Tu in *E. Coli*

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Two copies of the structural gene for the elongation factor EF-Tu have been identified in Escherichia coli: one near rif and the other near str. The latter seems to belong to a single transcriptional unit together with the genes for ribosomal protein S7, S12 (str) and the elongation factor EF-G (fus).

PROTEIN chain elongation factors, EF-G, EF-Tu and EF-Ts, have been characterised as components required for protein synthesis in *Escherichia coli* (for reviews, see refs 1-3). In addition to their role in protein synthesis, both EF-Tu and EF-Ts are known to be components of Q β -replicase⁴. Furthermore, EF-Tu has been suggested to function as a positive regulatory factor for the transcription of rRNA genes^{5,6}. Since these protein chain elongation factors, especially EF-G and EF-Tu, function in close association with the ribosome, one could reasonably expect that their amounts inside a cell would be regulated coordinately with the amount of ribosomes. Such coordinated regulation has been observed for EF-G and EF-Ts (refs 7 and 8). No study has, however, been reported on the regulation of EF-Tu (see ref. 8).

To study the regulation of synthesis of the elongation factors, information on the organisation of the genes for these factors is essential. The gene for EF-G has been identified and mapped^{9,10} at 64 min near the *str* locus that codes for ribosomal protein (r-protein) S12, but no information was available concerning the genes for EF-Tu and EF-Ts. In this paper, we report the identification of the gene for EF-Tu. It was found that there are at least two copies of the gene for EF-Tu on the haploid chromosome of *E. coli*; one is located close to the *str* gene at 64 min and the other is at 79 min close to the *rif* gene which codes for the β -subunit of RNA polymerase³⁹. In addition, the former gene seems to belong to a single transcriptional unit together with the genes for EF-G (*fus*), r-protein S12 (*str*), and S7.

$\lambda fus2$ and λrif^{d18} carry EF-Tu genes

Several λ transducing phages carrying many r-protein genes from the *str-spc* region of the *E. coli* chromosome at 64 min have been isolated previously¹¹. One of them, $\lambda fus2$, carries about 30 r-protein genes, and in addition, the *fus* gene which codes for EF-G. In addition to the *str-spc* region, several 50S r-protein genes are also located near *rif* at 79 min. It has been found^{12,40} that λrif^{d18} , originally isolated by Kirschbaum and Konrad¹³, carries several 50S r-protein genes in addition to the genes for RNA polymerase subunits β and β' (ref. 14). Because of the close relationship between elongation factors and ribosomes, we have investigated the possibility that genes for EF-Tu and EF-Ts might be located close to the r-protein genes carried by $\lambda fus2$ (or λrif^{d18}) as is the case with the *fus* gene.

First, ultraviolet-irradiated *E. coli* cells were infected with purified transducing phages, and proteins synthesised after phage infection were labelled with ³⁵S-methionine and analysed

(see ref. 11, and the legend to Fig. 1). Ultraviolet irradiation damages bacterial DNA and decreases the synthesis of RNA and protein to a negligible level without serious damage to the RNA and protein synthesis machinery. Thus, large stimulation in the synthesis of any protein observed after introduction of intact transducing phage DNA is a strong indication of the presence of a structural gene for that protein on the transducing phage DNA. A λ lysogen was used to prevent the expression of λ genes on the infecting transducing phages. The proteins synthesised after infection with the transducing phages were analysed by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis¹⁵ followed by autoradiography (Fig. 1).

It can be seen that $\lambda fus2$ stimulated the synthesis of several proteins. Comparison to the reference proteins indicated the presence of a radioactive protein band corresponding to the EF-G band as expected. The presence of EF-G in this band was confirmed by tryptic peptide analysis (data not shown). In addition, we found radioactive protein bands with the mobilities of the reference EF-Tu (see below) and the reference RNA polymerase subunit α (S.R.J., R.R.B., and M.N., unpublished). In contrast to $\lambda fus2$, neither $\lambda spc2$ nor $\lambda spc1$ stimulated the synthesis of the proteins corresponding to EF-G and EF-Tu. $\lambda spc2$ and $\lambda spc1$ are other transducing phages carrying r-protein genes including *spc*, but not *fus* (see ref. 11 and Fig. 5). To our surprise, however, we found that λrif^{d18} also stimulated the synthesis of a radioactive protein which migrated like EF-Tu on the SDS-gel system used.

The radioactive proteins with the mobility of EF-Tu were further characterised. These proteins were synthesised after $\lambda fus2$ or λrif^{d18} infection in the presence of ³⁵S-methionine. We call them $\lambda fus2$ -EF-Tu and λrif^{d18} -EF-Tu, respectively. The following observations were made. (1) As described above, both $\lambda fus2$ -EF-Tu and λrif^{d18} -EF-Tu showed the same mobility in the SDS-polyacrylamide gel electrophoresis as the reference EF-Tu (see Fig. 1b). (2) Both proteins also showed the same mobility in another polyacrylamide gel electrophoresis system (pH 8.7, in 8 M urea) as the reference EF-Tu (data not shown). (3) Both $\lambda fus2$ -EF-Tu and λrif^{d18} -EF-Tu labelled with ³⁵S-methionine were purified by SDS-polyacrylamide gel electrophoresis and then digested with trypsin. The resultant methionine-containing peptides were compared with those obtained from a reference EF-Tu purified from cells labelled with ³⁵S-methionine. Radioactive methionine containing tryptic peptides were analysed by paper electrophoresis at pH 3.6. As Fig. 2 shows, both radioactive proteins, $\lambda fus2$ -EF-Tu and λrif^{d18} -EF-Tu, in fact showed a tryptic peptide pattern identical to that of reference EF-Tu. (4) Both radioactive proteins reacted with a specific antiserum against purified EF-Tu, but not with an antiserum against EF-G (data not shown). From these results, we conclude that both $\lambda fus2$ and λrif^{d18} stimulated the synthesis of EF-Tu in the ultraviolet-irradiated *E. coli* cells.

We have also demonstrated that the DNAs from $\lambda fus2$ and λrif^{d18} are both active in the synthesis of EF-Tu in an *in vitro* DNA-dependent protein synthesising system described previously¹⁶. As described in Fig. 3, radioactive proteins synthesised

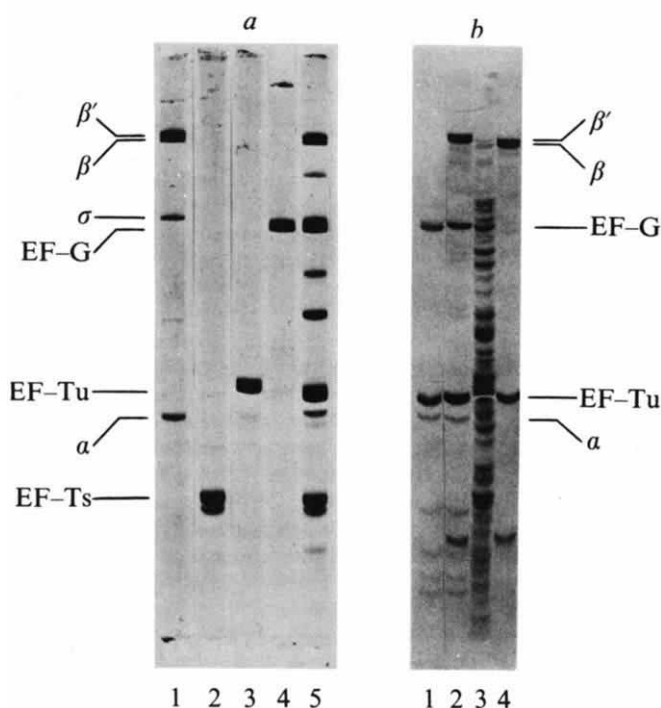


Fig. 1 Electrophoresis of proteins synthesised in ultraviolet-irradiated bacteria after infection with transducing phage. *a*, Reference proteins were electrophoresed on 8.75% polyacrylamide disc gels containing 0.1% sodium dodecyl sulphate (SDS) as described previously¹⁸. The samples were: (1) RNA polymerase; (2) EF-Ts; (3) EF-Tu; (4) EF-G; (5) a mixture of RNA polymerase, EF-Ts, EF-Tu, EF-G and several molecular weight markers. Gels were stained with Coomassie brilliant blue. The bands are identified on the left. *b*, Infection of ultraviolet-irradiated *E. coli* cells with transducing phages was performed essentially as described previously¹¹. The host was a λ lysogen of *E. coli* K12 strain 159 (ref. 32). The culture medium was a synthetic minimal medium³³ containing maltose (0.4%) and B1 (1 $\mu\text{g ml}^{-1}$). The multiplicity of infection was approximately 10. For each sample, 5 μCi of ^{35}S -methionine (323 Ci mmol^{-1}) was added to a 2.5 ml culture 30 min after infection. After a 10 min labelling period and a 1-min chase with non-radioactive methionine (1 mg ml^{-1}), the culture was diluted with 5 ml of ice-cold minimal medium without maltose. The cells were pelleted, washed once with 0.05 M Tris-HCl (pH 7.9), and stored at -20°C . Cell pellets were resuspended in 250 μl SDS-gel sample buffer [3% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 10% (v/v) glycerol, 0.063 M Tris-HCl pH 6.8], heated for 2 min at 90°C , and 25 μl electrophoresed in parallel with the reference proteins shown in (*a*). The stained and destained gels were sliced longitudinally, dried down under vacuum on to a piece of filter paper, and subjected to autoradiography using Kodak No-Screen X-ray film. The autoradiogram shown was exposed for 4 d. The samples were ultraviolet-irradiated cells infected with: (1) $\lambda\text{fus}2$; (4) λrif^{d18} . Gel 2 contained a 1:1 mixture of the samples applied to gels 1 and 4. The sample for gel 3 was uninfected non-irradiated cells labelled with ^{35}S -methionine. The ultraviolet-irradiated cells infected with $\lambda\text{c1857S7}$ did not show any radioactive band (compare sample 1 in Fig. 4).

in vitro using $\lambda\text{fus}2$ DNA and λrif^{d18} DNA as template were first analysed by SDS-polyacrylamide gel electrophoresis. The radioactive proteins with the mobility of EF-Tu were detected in both cases. These proteins were eluted from the gels and examined for their reaction to specific antisera against EF-Tu, EF-G and r-protein L1, respectively. As shown in Fig. 3, radioactive proteins synthesised with $\lambda\text{fus}2$ DNA and λrif^{d18} DNA as template both reacted with the anti-EF-Tu serum but not with the other two antisera used. These experiments demonstrate that a protein very similar or identical to the reference EF-Tu was synthesised using either $\lambda\text{fus}2$ DNA or λrif^{d18} DNA as template. From all these experimental results, we conclude that both $\lambda\text{fus}2$ and λrif^{d18} carry a structural gene for EF-Tu.

In preliminary experiments, we have attempted to find a gene for EF-Ts on either the $\lambda\text{fus}2$ or λrif^{d18} chromosome, but with negative results.

Transcriptional unit containing *str*, *fus* and an EF-Tu gene

We have isolated several mutants of $\lambda\text{fus}3$ that have a decreased expression of the EF-Tu gene in ultraviolet-irradiated cells. $\lambda\text{fus}3$ is a derivative of $\lambda\text{fus}2$ which carries a *str*^s allele instead of the *str*^r allele present on $\lambda\text{fus}2$. We started from a lysogen (NO1380) carrying $\lambda\text{fus}3$ and with chromosomal markers *recA*⁻, *trkA*⁻, *spc*^r, *str*^r and *fus*^r. Since the *spc*^s, *str*^s and *fus*^s alleles carried by $\lambda\text{fus}3$ are dominant over the corresponding resistant alleles on the chromosome, the phenotype of the lysogen is sensitive to all three antibiotics, that is, streptomycin, spectinomycin and fusidic acid (Str-S, Spc-S and Fus-S). We selected spontaneous Str-R mutants on media with a low potassium concentration and containing Str. By using media with a low potassium concentration, we avoided selecting mutants that had simply lost the transducing phage since the *trkA*⁺ gene on $\lambda\text{fus}3$ is required for growth on low potassium media¹⁷. Any mutation which inactivates the expression of the

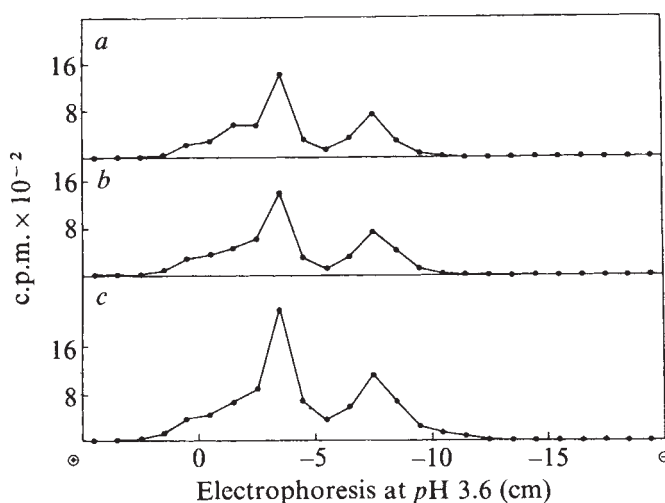


Fig. 2 Electrophoresis of ^{35}S -methionine tryptic peptides of EF-Tu. The three samples containing ^{35}S -methionine labelled EF-Tu described below were electrophoresed with 1 μg pure EF-Tu as described in Fig. 1. The stained band corresponding to EF-Tu sized protein was cut out from each gel, washed in water, crushed, equilibrated with 0.1 M NH_4HCO_3 , and incubated for 24 h at 37°C with 100 $\mu\text{g ml}^{-1}$ TPCK treated trypsin. Solutions were separated and the gel particles were again treated with trypsin. The solutions from the two trypsin treatments were pooled. The peptides eluted into the solution were recovered by lyophilisation and electrophoresed on Whatman 3 MM, 25 V cm^{-1} , for 2.5 h with H_2O , glacial acetic acid, pyridine (90:10:1) at pH 3.6. The origin is at 0. Strips (1-cm) were counted by liquid scintillation counting. The samples were: *a*, The EF-Tu sized protein synthesised in ultraviolet-irradiated cells after infection with λrif^{d18} ; *b*, the EF-Tu sized protein synthesised in ultraviolet-irradiated cells after infection with $\lambda\text{fus}2$; *c*, reference EF-Tu. The source of the reference EF-Tu was as follows. First, a partially purified EF-T preparation was made from uninfected non-irradiated *E. coli* cells [strain 159(λ)] grown in the presence of ^{35}S -methionine. The purification was done according to the previously described procedures^{34,35} as modified by W. A. Strycharz (personal communication). The cells were broken with alumina and a S100 supernatant was treated with protamine sulphate and fractionated with ammonium sulphate and isopropanol³⁴. The protein fraction containing EF-T was applied to a DEAE-Sephadex column³⁵. The column was first washed with 0.15 M KCl; EF-T was then eluted with 0.30 M KCl. The partially purified EF-T preparation thus obtained was subjected to electrophoresis on an SDS-polyacrylamide gel. The electrophoresis separated EF-Tu from Ts and all other contaminating proteins in the EF-T preparation. Pure EF-Tu prepared in this way was used as the reference EF-Tu.

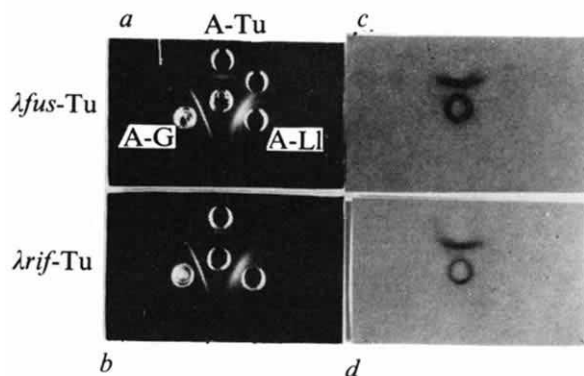


Fig. 3 Identification by radioimmunodiffusion of EF-Tu synthesised *in vitro*. ^{35}S -methionine-labelled protein was synthesised in a DNA-dependent cell-free system, using DNA from $\lambda fus2$ ($35 \mu\text{g ml}^{-1}$) and that from λrif^{18} ($40 \mu\text{g ml}^{-1}$) as template. The method was similar to those described previously^{11,16}. After incubation at 37°C for 90 min, samples were treated with RNase ($10 \mu\text{g ml}^{-1}$) and DNase ($10 \mu\text{g ml}^{-1}$) at 37°C for 10 min. The resultant samples ($60 \mu\text{l}$ each) were mixed with $200 \mu\text{l}$ of the SDS-gel sample buffer (see Fig. 1), heated at 95°C for 5 min, cooled and dialysed against 4 changes of 50 ml of the SDS-gel sample buffer³⁸. The samples were then subjected to the SDS-polyacrylamide gel electrophoresis as described in Fig. 1 and the radioactive products were detected by autoradiography. The radioactive proteins which had mobilities identical to reference EF-Tu were eluted from the sliced gels. This was done by crushing the gels and incubating them with 1.5 ml of $0.1 \text{ M NH}_4\text{HCO}_3$ ($\text{pH} \sim 8.0$) for 20 h at 37°C . Bovine serum albumin ($50 \mu\text{g}$) was added to the eluates and the mixtures were lyophilised. Lyophilised proteins were then dissolved in $50 \mu\text{l}$ of Tris-HCl (0.03 M , $\text{pH} 7.4$)-MgCl₂ (0.02 M)-KCl (1 M)-2-mercaptoethanol (0.006 M) buffer containing the following carrier proteins: purified T (EF-Tu and EF-Ts), $3 \mu\text{g}$; purified EF-G, $2 \mu\text{g}$; unfractionated 50S ribosomal proteins, $30 \mu\text{g}$. These protein mixtures containing radioactive proteins were analysed by immunodiffusion as described before^{16,38}. The samples were placed in the centre wells and antisera against EF-Tu ("A-Tu"), EF-G ("A-G") (both gifts from H. Weissbach) and 50S protein L1 ("A-L1")¹² were placed as indicated in the figure (both in a and b). Each set of pictures shows a gel (a and b) and an autoradiogram of the dried gel (c and d). The results obtained with the sample containing the *in vitro* product using $\lambda fus2$ DNA as template are shown in a and c and those obtained using λrif^{18} DNA are shown in b and d. It can be seen in both cases that only the Tu-anti-Tu precipitin bands contained radioactive proteins and the other precipitin bands did not.

str^s gene on $\lambda fus3$ or changes it to a *str^r* allele will lead to the Str-R phenotype. Among 25 independent Str-R mutant lysogens isolated, 15 were Fus-R (but not Spc-R). Transducing phages were isolated and characterised. Phages that seemed to have a mutation inactivating the *str^s* and *fus^s* genes were further analysed for their ability to stimulate the synthesis of EF-G, EF-Tu and r-proteins. Table 1 and Fig. 4 give the results obtained with two mutant transducing phages, $\lambda fus3\Delta 101$ and $\lambda fus3\text{-I103}$. As can be seen, these two mutations substantially reduce the expression of the genes for EF-G, EF-Tu, S12 (*str*), and S7. It should be noted that the expression of these genes does not seem to be completely eliminated. For example, a small amount (about 3% of the parent) of EF-G seems to be made from $\lambda fus3\Delta 101$ (see Fig. 4). This suggests that the inactivation of some or all of these genes is not a direct inactivation such as that caused by a deletion.

The mutations carried by these two transducing phages, $\Delta 101$ and I103, were characterised by the density of the mutant phages and by electron microscopic analysis of heteroduplexes of the mutant phage DNAs and several other phage DNAs including the $\lambda fus2$ DNA. We have found that mutation I103 is an insertion of a small DNA with the size of IS2 (refs 18–20) and is located close to the right end of the bacterial genome carried by $\lambda fus3$ (see Fig. 5). The distance between the site of the insertion and the right *coli*- λ junction was found to be 0.60 ± 0.10 kilobase (kb) in length. Mutation $\Delta 101$ was found to be a

small deletion which is also located near the right end of the $\lambda fus3$ chromosome. This deletion removes the *coli*- λ junction; it deletes about 0.3 kb of λ DNA together with about 1.3 kb of the right end of the bacterial DNA (Fig. 5). The sum of the minimum probable sizes of each of the inactivated genes, that is, the structural genes for EF-G, EF-Tu, S12 and S7, can be calculated from the molecular weights of these proteins. The sum is 4.6 kb in length, and is much larger than the bacterial DNA removed by $\Delta 101$ or the space between the right "*coli*- λ " junction and the site of the I103 insertion. Thus, most of the inactivated genes seem to be located left of the mutations $\Delta 101$ or I103. We conclude that most of the genes for EF-G, EF-Tu, S12 (*str*) and S7 are not deleted, but inactivated by some kind of polar effect. Since the direction of the transcription in this region is from right to left²¹, and there is no known λ promoter on the λ DNA present on $\lambda fus3$, the transcription of these genes seems to be from a bacterial promoter which would be located near the right end of the bacterial DNA. Thus, the polar effect of $\Delta 101$ is probably caused by the deletion of the promoter or some other essential regulatory elements for these inactivated genes. Similarly, inactivation of these genes by I103 is probably

Table 1 Relative synthesis of protein in ultraviolet-irradiated bacteria after infection with $\lambda fus3$, $\lambda fus3\Delta 101$, $\lambda fus3\text{-I103}$

Protein	$\lambda fus3$	Transducing phage $\lambda fus3\Delta 101$	$\lambda fus3\text{-I103}$
EF-G	1.0	0.03	0.19
EF-Tu	1.0	0.11	0.23
S7	1.0	0.31	0.34
S12	1.0	0.03	0.13
Others	1.0	1.02	0.99

Ultraviolet-irradiated bacteria were infected with $\lambda c1857\text{S7}$, $\lambda fus3$, $\lambda fus3\Delta 101$ and $\lambda fus3\text{-I103}$ as described in Fig. 1. The relative amounts of EF-G and EF-Tu synthesised after phage infection were determined from the autoradiogram shown in Fig. 4. The autoradiogram was scanned with a Joyce-Loebl microdensitometer and the amounts of EF-G and EF-Tu synthesised relative to the amount of RNA polymerase subunit α synthesised were calculated from the areas of the peaks. The synthesis of α does not seem to be affected by the $\Delta 101$ and I103 mutations. Its structural gene is between *trkA* and *spc* (S.R.J., R.R.B. and M.N., unpublished). The amount of each protein synthesised was then normalised to the amount synthesised after infection with the parent phage $\lambda fus3$. The relative synthesis of S7 and S12 was determined from separate experiments in which the labelling and sample preparation were done as described previously¹¹. The bacterial host for the experiment was prelabelled with ^{14}C -leucine, irradiated, and infected with transducing phages. Proteins synthesised after infection were labelled with ^3H -leucine and electrophoresed on a two-dimensional polyacrylamide gel that separates r-protein³⁰. The $^3\text{H}/^{14}\text{C}$ ratio for all the r-proteins except S1, L31 and L34 was determined. The stimulation of each r-protein i was calculated, $S_{rpi} = (^3\text{H}/^{14}\text{C})_{\text{transducing phage infected}} / (^3\text{H}/^{14}\text{C})_{\lambda c1857\text{S7 infected}}$. The stimulation of the synthesis of each r-protein relative to the synthesis of S4 was then determined by dividing S_{rpi} by the stimulation of the synthesis of S4, S_{rpi}/S_{rps4} . The structural gene for S4 is between *trkA* and *spc* (refs 21 and 31) and its synthesis does not seem to be affected by the $\Delta 101$ and I103 mutations. The relative stimulation of the synthesis of each protein by $\lambda fus3\Delta 101$ and $\lambda fus3\text{-I103}$ was then normalised to the relative stimulation by $\lambda fus3$: $(S_{rpi}/S_{rps4})_{\text{transducing phage infected}} / (S_{rpi}/S_{rps4})_{\lambda fus3 \text{ infected}}$. Data for S7 and S12 are presented here. The stimulation of the synthesis of some protein that comigrates with L20 is also reduced to less than 10% by $\Delta 101$ and I103. Since some S12 comigrates with L20 (ref. 30 and M.N., unpublished), it seems likely that the apparent stimulation of the synthesis of L20 by $\lambda fus3$ and its reduction by $\Delta 101$ and I103 is due to the presence of S12 in this spot. The stimulation of the synthesis of each of the other r-proteins whose genes appear to be on $\lambda fus3$ (27 proteins) was not significantly affected. The average of the normalised relative stimulation for these proteins is given in the bottom line. We previously observed an apparent weak stimulation of the synthesis of S7 in ultraviolet-irradiated cells after infection with $\lambda spc1$ and $\lambda spc2$ (ref. 11). The stimulation of the synthesis of S7 is, however, much greater with $\lambda fus3$ or $\lambda fus2$ (S.R.J. and M.N., unpublished). Also, we have only been able to synthesise S7 *in vitro* using $\lambda fus2$ DNA as template and not with $\lambda spc1$ or $\lambda spc2$ DNA (L.L. and M.N., unpublished). Thus it is likely that the structural gene for S7 is present on $\lambda fus2$ and $\lambda fus3$, but not on $\lambda spc1$ or $\lambda spc2$.

due to a strong polar effect affecting distal genes that is analogous to the polar effect of IS2 studied in other systems¹⁸⁻²⁰. From these results, we conclude that the genes for EF-G (*fus*), EF-Tu, S12 (*str*) and S7 probably belong to a single transcriptional unit. The promoter for this transcriptional unit is presumably located in the region between the I103 insertion and the *coli*- λ junction, the region that is also covered by the Δ 101 deletion. More complicated models, however, such as the expression of one transcriptional unit being required for the expression of another unit, would also be consistent with present observations.

Because the mutations Δ 101 and I103 affect the four genes described above, but do not affect the remaining r-protein genes, including *spc* or the gene for the RNA polymerase subunit α , these latter genes presumably belong to another one or more transcriptional units. This is consistent with the previous conclusion that there is more than one r-protein gene transcriptional unit in the *str*-*spc* region of the *E. coli* chromosome²¹. As mentioned above, EF-Tu is synthesised in ultraviolet-irradiated cells after λ *fus2*, but not after λ *spc1* or λ *spc2*, infection. These results suggest that the gene for EF-Tu is not on the λ *spc2* genome, placing the gene location as shown in Fig. 5. This mapping position is consistent with the conclusion described above that the gene for EF-Tu is in the same transcriptional unit as *str* and *fus*.

EF-Tu gene in *rif* region

The EF-Tu gene has been mapped on the λ *rif*^{d18} chromosome by making DNA fragments with various DNA restriction endonucleases and testing the ability of each fragment to synthesise EF-Tu in a DNA-dependent protein synthesising system (L.L., M. Yamamoto and M.N., unpublished). The order of the genes is shown in Fig. 5. We do not have any experimental information on the question of whether the EF-Tu gene is cotranscribed together with any of the neighbouring genes, the rRNA gene set¹², 50S r-protein genes^{12,40} or the genes for the RNA polymerase subunits β and β' (refs 14, 22 and 23).

Two genes for EF-Tu

We believe that the presence of the genes for EF-Tu on both λ *rif*^{d18} and λ *fus2* reflects the presence of at least two genes for EF-Tu on the normal *E. coli* chromosome. Since the gene for EF-Tu was not used as a selection marker to isolate the λ *rif*^{d18} or λ *fus2* transducing phages, it is difficult to imagine that the EF-Tu gene was accidentally incorporated into these transducing phages during construction of the phage. In addition, we have observed that another transducing phage, ϕ 80*rif*^r, isolated by Konrad, Austin and Kirschbaum²⁴, also stimulates synthesis of EF-Tu, in addition to RNA polymerase subunits β and β' , and several 50S r-proteins, in ultraviolet-irradiated cells (our unpublished experiments). It was also shown that the DNA of ϕ 80*rif*^r has a near complete homology to λ *rif*^{d18} phage DNA in the substituted bacterial DNA region containing the 50S r-protein genes, the EF-Tu gene and the rRNA gene set, as judged by electron microscopic analysis of the heteroduplex formed from both the phage DNAs (G.D. Strycharz, and M.N., unpublished.) Since ϕ 80*rif*^r was originally isolated in a completely different way from that used for the λ *rif*^{d18} isolation, this observation also supports the conclusion that the organisation of the genes found on the λ *rif*^{d18} phage chromosome applies to the normal *E. coli* chromosome. We conclude that there are at least two genes for EF-Tu. We call the one near *str* and *fus* "tufA" and another near *rif* "tufB".

There are some reports on the isolation of mutants which have alterations in EF-Tu (refs 25 and 37). The genes responsible for the alterations have not, however, been mapped.

The significance of the presence of two genes for EF-Tu, one at the *str*-*spc* region and the other at the *rif* region, is a matter of speculation at the moment. The two chromosomal regions

where the *tuf* genes are located contain many of the genes for ribosome and RNA polymerase components. One could speculate that these two regions are physically proximate within *E. coli* cells and that the transcription and translation of these genes as well as the assembly of both RNA polymerase and ribosomes take place all in one place. It is possible that a physical interaction (by DNA sequence homology) between the two *tuf* genes is involved in maintaining a chromosome structure with the two regions in contact. A condensed *E. coli* chromosome structure with several "contact regions" has been proposed recently²⁶.

Alternatively, EF-Tu might function as a regulatory protein for the expression of its own operon. If such regulation takes place using a "nascent form" of EF-Tu and it acts only *cis* (that is, only on a regulatory element of its own operon), the presence of one EF-Tu gene for each important gene cluster, one at the *rif* region and the other at the *str*-*spc* region, would be required for the proper regulation.

It is also possible that the two observed *tuf* genes may be slightly different and may code for two kinds of EF-Tu that have slightly different primary structures and different cellular functions. It is equally conceivable that the presence of the two *tuf* gene copies may simply reflect a need for a greater amount of EF-Tu for cellular functions. In this connection, we note that although the stoichiometric relationship of both EF-G and EF-Ts to ribosomes has been analysed^{7,8,27}, no such work

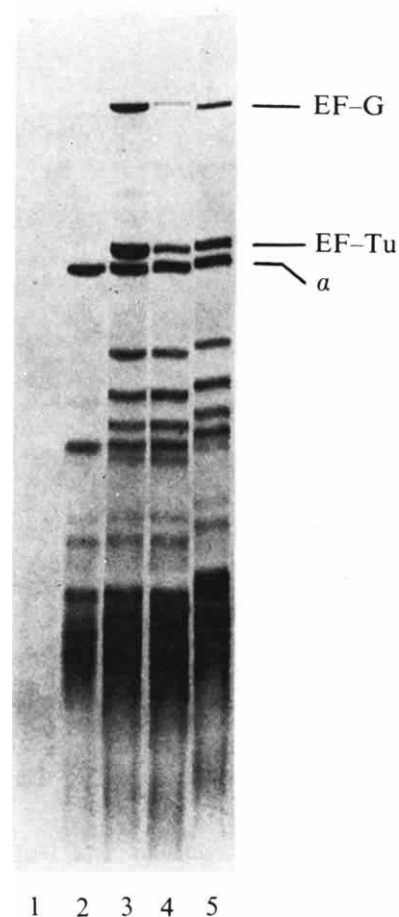
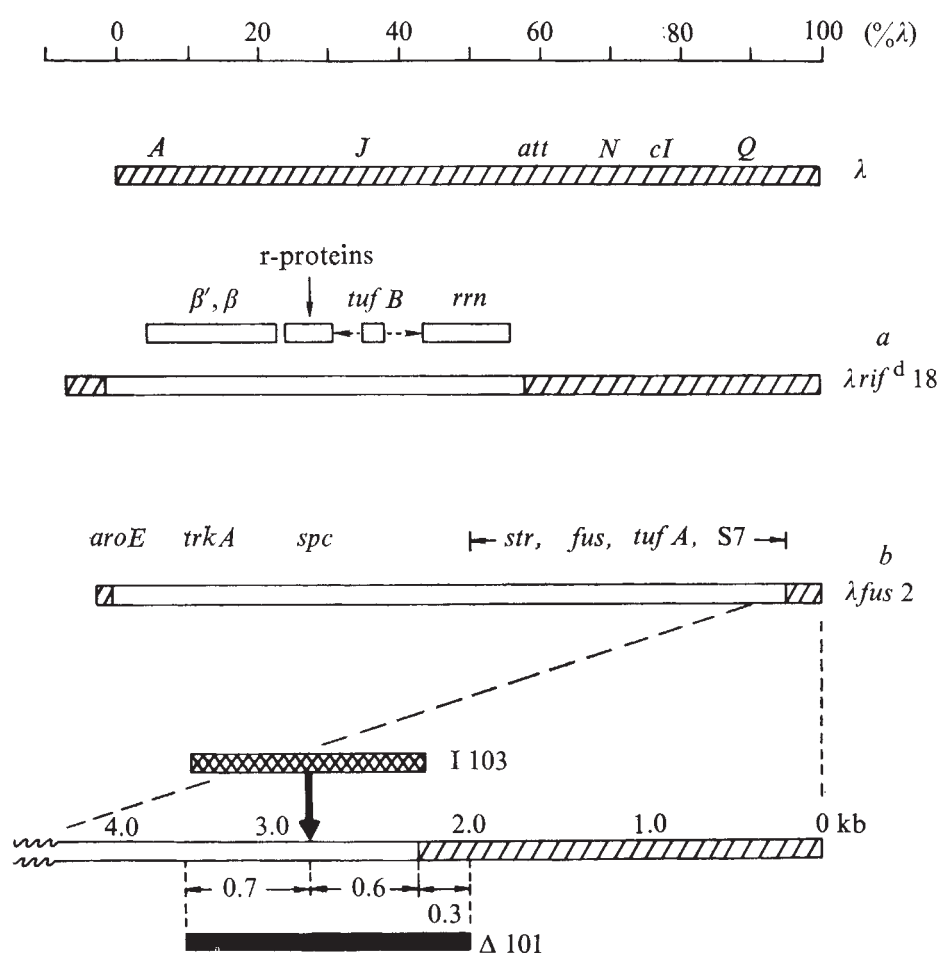


Fig. 4 Electrophoresis of proteins synthesised in ultraviolet-irradiated bacteria after infection with mutant transducing phages. Ultraviolet-irradiated cells were infected with (1) λ c1857S7, (2) λ *spc2*, (3) λ *fus3*, (4) λ *fus3*- Δ 101, and (5) λ *fus3*-I103 as described for Fig. 1. Samples were prepared and analysed by SDS-polyacrylamide gel electrophoresis as described in Fig. 1 except that the gels contained 12.5% acrylamide instead of 8.75%. Autoradiograms of dried gels are shown. Some of the bands are identified on the right.



to 48.5% λ measured from the left end of λ genome, and inserts a 50.7% λ units length fragment of bacterial DNA; λ spc1 deletes λ DNA from 1.9% λ to 56.8% λ and inserts 38.4% λ units length fragment of bacterial DNA; λ spc2 deletes λ DNA from 1.9% λ to 94.9% λ and inserts 96.5% λ units length fragment of bacterial DNA. Thus, λ spc2 has a homology to the DNA segment from the left end of λ spc2 to the right end of the bacterial DNA segment in λ spc2 (total about 53% λ units) and a homology to the λ DNA at the end of the molecule with the length of 5.1% λ units. The structure of these transducing phages has been determined previously (M. Fiant, W. Szybalski, F. Blattner, L.L., S. R. J. and M.N., unpublished).

has been reported with respect to EF-Tu (see ref. 8).

Finally, we have shown that the genes for EF-G (*fus*) and that for EF-Tu (*tufA*) probably belong to a transcriptional unit together with other r-protein genes including *str*. Thus we expect that both the *fus* gene and the *tufA* gene are subject to the same control system as those governing r-protein genes, such as the stringent-relaxed control system^{28,29}. We do not, however, know whether the *tufB* gene is also controlled by the same system. Thus more experiments are required to clarify the problems related to the regulation of the *tuf* gene expression.

We thank Dr H. Weissbach for providing us with purified EF-G, EF-Tu and EF-Ts preparations and antisera against EF-G and EF-Tu; and H. Ahrens, K. Ryan, L. Sadowski, M. Stroud and G. D. Strycharz for technical assistance. This work was supported by grants from the US National Science Foundation and the National Institutes of Health (administered by M.N.) and the National Institutes of Health (administered by R.R.B.). L. L. is a recipient of an EMBO postdoctoral fellowship.

Received July 9; accepted August 25, 1975.

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Fig. 5 The structure of λ rif^d18 (a) and λ fus2 (b), showing the location of *tufA*, *tufB* and the mutations I103 and Δ 101. DNA from the λ genome is hatched. a, The location and the probable minimum sizes of the genes for β and β' , the genes for r-proteins (L1, L7/12, L8, L10 and L11), *tufB* (the gene for EF-Tu) and *rrn* (genes for 16S, 23S and 5S rRNA) are indicated above the λ rif^d18 chromosome. The location of *tufB* is between r-protein genes and *rrn* gene, but it is not exactly determined. The locations of genes for L1 and L7/12 have been experimentally determined and the genes for the other r-proteins are assumed to be located together with these genes ("r-proteins"). The map is based on the work of L.L., M. Yamamoto and M.N. (unpublished). b, The size and location of the insertion mutation I103 and the deletion mutation Δ 101 are indicated on the λ fus2 chromosome. (The structure of λ fus3 is identical to that of λ fus2. Approximate sizes of various regions are indicated in kilo bases (kb). The size of the standard λ genome is 46.5 kb.) In addition, the locations of various bacterial genes are indicated above the λ fus2 chromosome. The locations of the genes for S7, *str*, *fus* and the gene for EF-Tu, *tufA* (the order not determined) were determined from comparison of the structure of λ fus2 which carries these genes with that of λ spc2 which does not carry them. Several other r-protein genes are located in this region but do not seem to be affected by Δ 101 or I103. λ spc2 deletes λ DNA from 1.9% λ

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letters to nature

New radio map of Cassiopeia A at 5 GHz

THE radio source Cassiopeia A (Cas A) is believed to be the remnant of a type II supernova that occurred in our galaxy about 280 yr ago (refs 1 and 2). The radio remnant forms an irregular shell of radius $\sim 2'$, corresponding to ~ 1.6 pc at its distance of 2.8 kpc (ref. 2), which is broadly coincident with an incomplete shell of fast-moving optical knots¹. We present here a new radio map of the source with a resolution three times greater than has previously been achieved³.

During November 1974, Cas A was observed with the Cambridge 5-km telescope⁴ at a frequency of 5 GHz, giving half-power beamwidths of $2''$ in right ascension and $2.3''$ in

declination. A total of 128 interferometer spacings were used, so that the synthesised beamshape has its first grating response at $5'$ in right ascension and does not confuse the source. The effective sensitivity is determined by the general level of the side-lobes, which, from regions of the map outside the source, is estimated to have a brightness temperature of 50 K, about 1% of the peak brightness of the source. The sensitivity from thermal noise alone would be 15 K.

Figure 1 shows the map, with contours of the Stokes' parameters $I-Q$. Figure 2 is a radio photograph showing the finer details not visible at the contour interval (480 K) of Fig. 1. The areas of lower surface brightness are shown very clearly in this negative print, which has 128 separate grey levels.

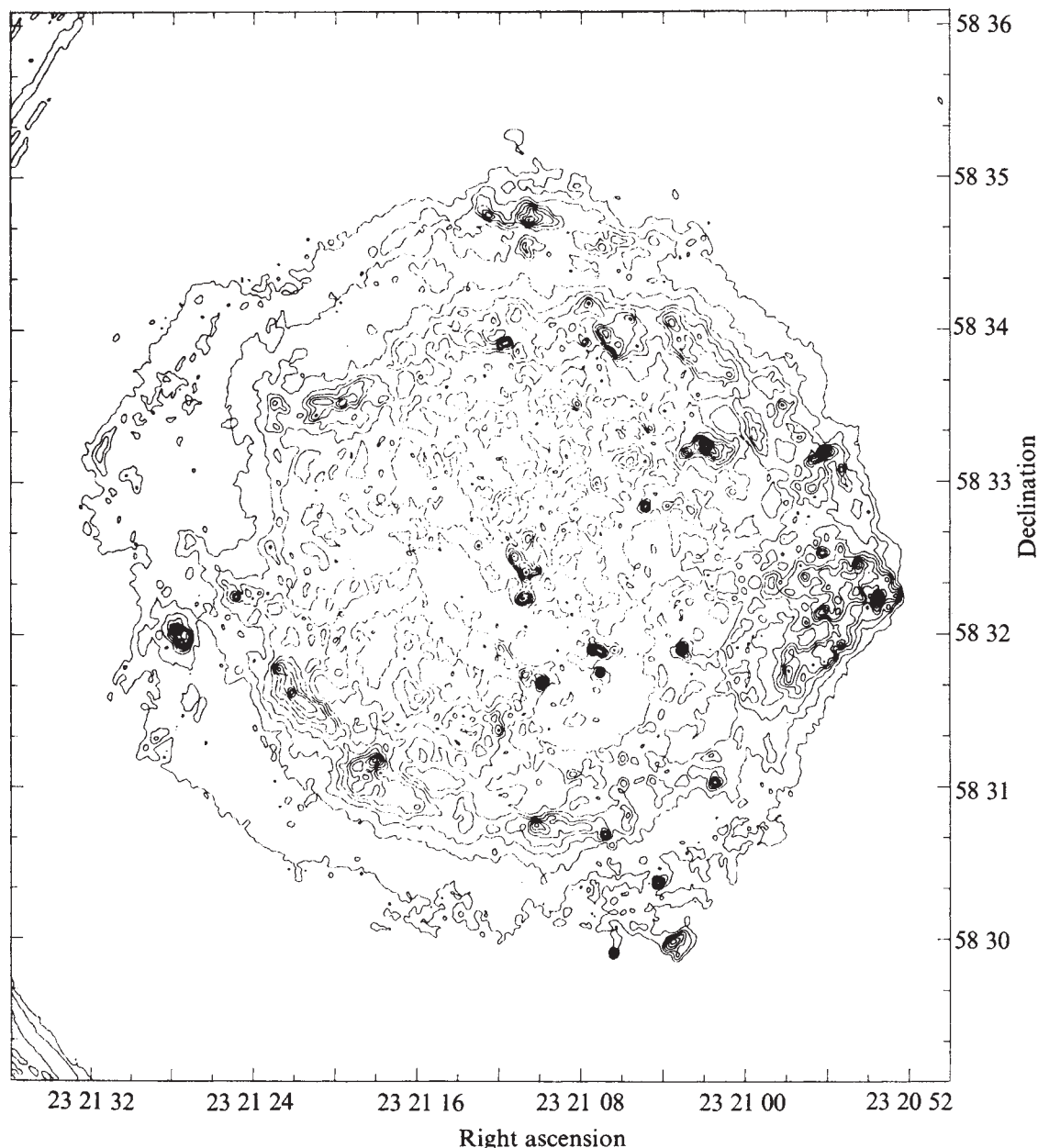


Fig. 1 Map of Cas A at 5 GHz. The contour interval is 480 K and the coordinates refer to epoch 1950.0



Fig. 2 Radio photograph of Cas A. The grey scales on either side are linear in radio intensity. The features visible at the corners of the map represent the first grating response.

The overall structure of the source is that of a ring, presumably the two-dimensional projection of a spherical shell. The observations show clearly that the brightest part of the ring of emission lies well inside the edge of the source, with a plateau of lower surface brightness outside, as has been noted previously³. This plateau itself shows detailed structure and in many areas seems to provide a well defined outer boundary to the remnant. The most plausible interpretation of these rather sharp boundaries is that they represent the position of a contorted, outer shock front and that relativistic electrons are able to diffuse as far as this shock. The extent of the plateau varies considerably over the remnant. In particular, there is no evidence of any plateau outside the strong peaks to the west of the source.

The ring of brightest emission shows considerable departures from circular symmetry. The well known gaps at position angle (p.a.) 70° and 210° (ref. 3) are less prominent at this resolution and are spanned by weak ridges of emission. There are several small, but clear-cut, gaps in the shell and one of these, at p.a. 200° ($\alpha = 23$ h 21 min 06.3 s, $\delta = 58^\circ 30'45''$), is of interest in relation to the bright knots of emission outside the main ring in this area. One of the new features revealed by the present observations is that the emission within the main ring shows a marked cellular structure defined by weak ridges on scales of $\sim 20''$; towards the edge of the source some of the cells seem flattened, suggestive of foreshortening. Such a cellular structure and the generally chaotic appearance of the remnant is in accord with models of young supernovae^{5,6}, in which the radio shells are first formed as a result of turbulence generated by the interaction between the ejected material and swept-up interstellar medium.

A notable feature of the remnant revealed by this map is the existence of many compact, strongly emitting regions on the scale of the beamwidth, particularly in the sector between p.a. 200° and 310°. Figure 3 is a more detailed contour map of part of the south-west region of the source and shows the proliferation of emission peaks in this area. Projection effects make it impossible to determine the position of most of these blobs relative to the main shell, but those outside

the shell to the south have clearly broken away from it. The radio photograph (Fig. 2) shows a V-shaped feature pointing towards these southern knots. Although this may merely be due to a chance coincidence of knots and filaments, it is reminiscent of a nozzle and we speculate that it represents the remains of that part of the shell from which the outer blobs detached, particularly as its axis coincides with the narrow gap in the bright ring of emission noted earlier. Another such nozzle can be picked out behind the strong, compact emission peaks at the extreme west of the source. Of the southern peaks, the small, slightly resolved, outermost knot at $\alpha = 23$ h 21 min 06.3 s, $\delta = 58^\circ 29'47''$ was not visible in 1969 (ref. 3) and it is intriguing that a recent plate taken by van den Bergh (personal communication) shows that an optical filament has now appeared in this position. This knot seems to have pulled out a neck of plateau in a non-radial direction and indeed all the outlying knots seem to have diffuse emission associated with them. There have also been many other significant changes both of flux and position since 1969 and a detailed study of these will be published elsewhere.

The minimum energy density in relativistic electrons and magnetic field required to account for the radio synchrotron emission from the most compact knots is $\sim 8 \times 10^{-8} \text{ J m}^{-3}$, equivalent to a magnetic field of $3 \times 10^{-7} \text{ T}$. If these knots are to be confined by the thermal pressure of hot, swept-up interstellar gas, then the product $n_H v_s^2$ must be greater than 14, where n_H is the local particle density in the interstellar medium (atom cm^{-3}) and v_s is the shock velocity (units of 1,000 km s^{-1}). Typical velocities of the fast optical filaments

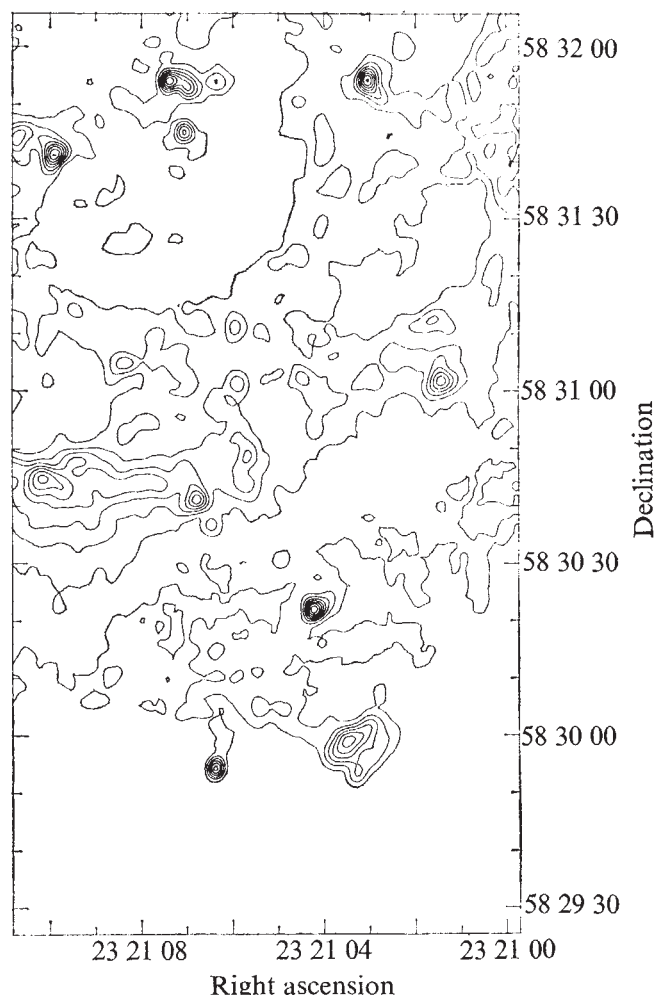


Fig. 3 Contour map of part of the south-west quadrant of Cas A. The contour level is 480 K.

are $5,000\text{--}7,000\text{ km s}^{-1}$, so containment is not difficult for reasonable values of n_H . Such high pressures, however, imply the existence of a very efficient mechanism for the production of magnetic field.

We thank Professor Sir Martin Ryle and the Staff of the Mullard Radio Astronomy Observatory for advice and assistance. We thank the Electron Microscopy Group of the Cavendish Laboratory, Cambridge, for the use of their Optonics 'Photowrite' System in the production of Fig. 2.

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Received August 11; accepted September 2, 1975.

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Observations at 6 cm of the solar active region

PRECISE knowledge of the fine structure of radio sources in solar active regions, and especially of their angular size and brightness temperature is important for the gyro-resonance absorption theory of the slowly varying component. In particular, such information is important for verifying proposed models^{1–3} of resonance absorption layers above sunspots for which measurement of circular polarisation is also extremely useful. The active regions at centimetre wavelengths remain stable in intensity and structure for periods of ~ 12 h. Consequently, the existing long baseline interferometers can be used to obtain synthesised maps from observations over those periods. Our first attempt to obtain such maps was in 1973 with the National Radio Astronomy Observatory interferometer at wavelengths of 3.7 and 11.1 cm. These maps were not very clean because of the availability of only three

baselines⁴, a problem which does not arise with the Westerbork synthesis radio telescope, which has twenty baselines. Here we describe the preliminary results of synthesis of a solar active region observed on May 8, 1974 from results obtained on May 8–10, 1974 with the Westerbork synthesis radio telescope (WSRT) in Holland⁵ at a wavelength of 6 cm.

The WSRT consists of twelve 25 m paraboloids along an east–west baseline, combined to form 20 interferometers. For our observations the minimum spacing was 90 m, the maximum 1,458 m, the separation between successive baselines was 72 m and the integration time was 30 s. At the wavelength of 6 cm and for a source with $\delta = 17^\circ$ the WSRT has a synthesised beam size of $6.3''$ (E–W) $\times$ $21.5''$ (N–S) and a primary beam with half width of $10'$. For a single 12-h period of observation the first grating ring has a radius of $3'$ (E–W) and $10'$ (N–S). In spite of the presence of several sources in the field of view, the associated grating rings were successfully removed from the maps by using conventional 'cleaning' techniques⁶. Because baselines smaller than 90 m are missing, the instrument eliminates a considerable fraction of the flux of sources with size greater than $1'$ (E–W) $\times$ $3'$ (N–S).

There are two major problems with a sidereal instrument used for solar observations: appropriate source tracking and receiver saturation. As the WSRT tracks at sidereal rate, we had to update the pointing of the dishes every 30 min. The difference between solar and sidereal motion over this interval (30 min) is about $0.6'$, which is comparable with the pointing error of the dishes. The phase drift of the visibility function due to solar motion was corrected in the data processing stage by a standard WSRT reduction routine. The accuracy of the tracking obtained this way is better than the synthesised beam width. Because of perspective changes caused by solar rotation during the 12-h observing period, however, only the region near the centre of the field of view can be tracked accurately. To avoid receiver saturation, an extra attenuation of about 25 dB was placed in front of the correlators. This attenuation was removed during calibration. The input of each correlator was adjusted to a constant level, the same for all correlators and all observations (solar and calibrator).

The instrument was used for 12 h of solar observation and 12 h of calibration each day. The sources 3C286, 3C147 and 3C48 were used for phase calibration and 3C147 for amplitude calibration. The accuracy of the phase calibration is better

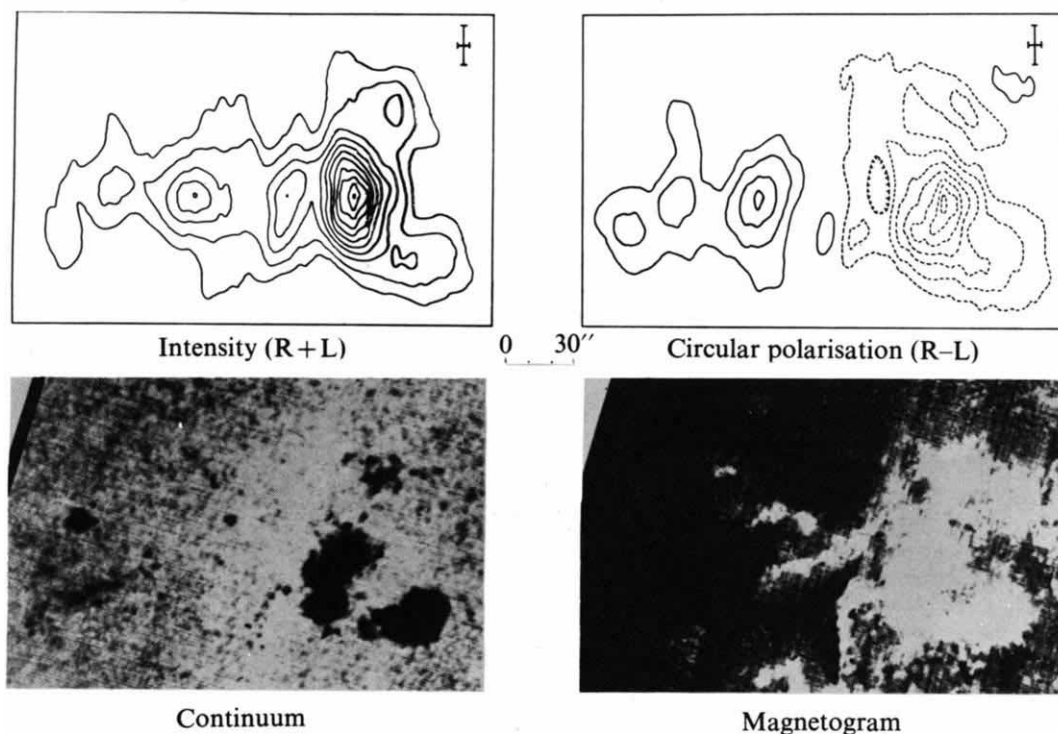


Fig. 1 Total intensity and circular polarisation maps of an active region near S12W05 on May 8, 1974. The beam size is shown in the upper right-hand corner. Contour levels are: 0.1, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50 ($\times 10^6$ K) for R+L; $-0.750, -0.625, -0.500, \pm 0.375, \pm 0.250, \pm 0.125, \pm 0.050$ ($\times 10^6$ K) for R–L. Negative contours are shown by broken contour lines. The hatched contour on the R–L map shows a decrease of the absolute value of the polarisation. Continuum photograph and magnetogram courtesy Sacramento Peak Observatory, AFCRL. Positive magnetic field is white.

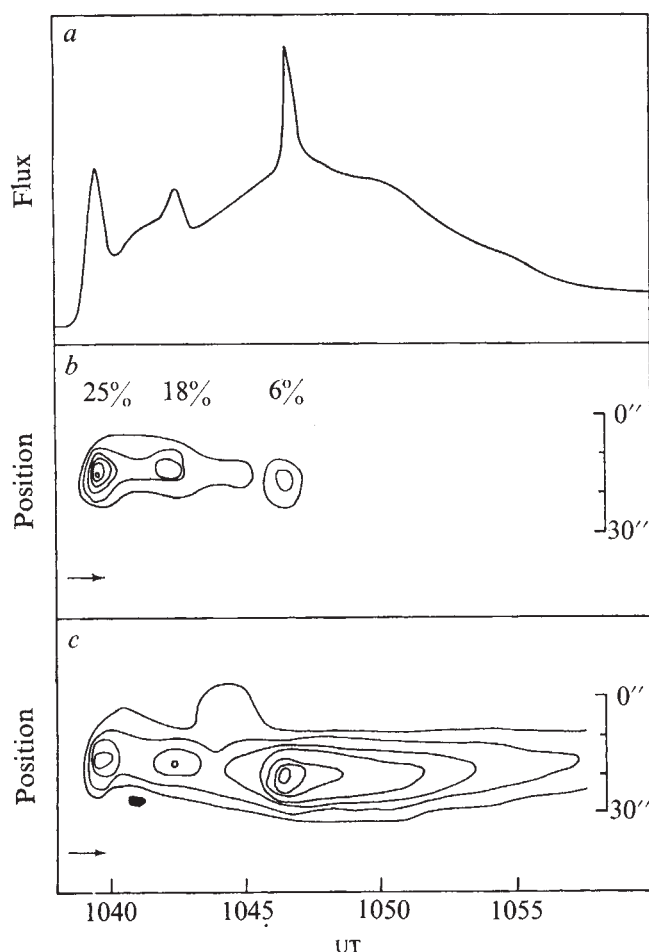


Fig. 2 A burst observed on May 9, 1974 with the WSRT: *a*, Flux (arbitrary units) as observed with a single Westerbork dish. *b*, Contours of equal circular polarisation (L-R) as a function of east-west position and time. Contour levels are: 0.29, 0.58, 0.87, 1.16, 1.45 and 1.74 ($\times 10^7$ K arc s). The peak values are 1.85×10^7 , 1.20×10^7 and 0.73×10^7 K arc s for the first, second and third peak respectively. The numbers indicate the percentage polarisation of the peaks. *c*, Equal intensity contours (L+R) as a function of east-west position and time. Contour levels are: 1.7, 3.4, 5.1, 6.8, 8.5, 10.2 and 11.9 ($\times 10^7$ K arc s). The peak values are 7.1×10^7 , 6.9×10^7 and 12.1×10^7 K arc s for the first, second and third peak respectively. The arrows in *b* and *c* show the position of the associated active region at S07W37. The response of constant sources has been subtracted from *b* and *c*.

than 1° , and the amplitude calibration is accurate to about 4%. The amplitude error is higher than the customary 1% in other WSRT observations because of the extra attenuation.

The data were calibrated and Fourier transformed by using standard WSRT reduction programs. The resulting 'dirty' maps were cleaned of sidelobes at the University of Maryland. All four Stokes parameters were observed, but the linear polarisation maps have a very noisy appearance, which puts the linear polarisation limit at about 2%. A total intensity and circular polarisation map from May 8 is shown in Fig. 1, together with a white light photograph and a magnetogram from Sacramento Peak Observatory. The coordinates of the sunspot group at 0 UT were S12W05. This group is part of McMath region 12906, which had a very extended plage.

Several individual sources show up in both total intensity and circular polarisation maps. The brightest source with a peak temperature of 2.5×10^6 K corresponds to one of the two large sunspots. The emission is extended northwards to the group of small sunspots, south-west towards the other large sunspot and eastward towards another group of small sunspots. The other three sources are overlying the plage with the brightest component located close to the neutral

line of the magnetic field. A low brightness halo surrounds the sources. The halo has a brightness temperature of about 10^5 K. It does not, however, extend over the entire plage region. This is probably because the interferometer spacings less than 90 m were not available for our observation.

As shown in the magnetogram, the active region has a bipolar structure with positive polarity concentrated on the main sunspots and negative polarity scattered over the plage and some minor sunspots. H α photographs show filaments along the neutral line. The circular polarisation map closely follows this configuration, with regions of positive magnetic polarity having the strongest degree of left circular polarisation. It is interesting to note that the source just to the east of the main peak lies along a neutral line and has a bipolar circular polarisation structure.

During our three days of observations several bursts were recorded. Because of the short lifetime of the bursts, it is not possible to produce a two-dimensional map with the interferometer. But, as the 20 baselines of the WSRT are located along a straight line, it is easy to combine them and produce the equivalent of a fan beam scan. This one-dimensional intensity distribution is displayed in Fig. 2 for a burst observed on May 9. We have used a contour map display, with the intensity shown as a function of position and time. For the sake of clarity we have subtracted the response of the constant sources. Our resolution is about $6''$ in the east-west direction; the integration time is 30 s. Figure 2 also gives the flux of the burst as recorded by a single Westerbork dish.

This particular event corresponds to an H α flare⁶ of importance 1B at S05W38. The flare occurred near a sunspot group shown by an arrow in Fig. 2. The burst shows three distinct maxima, the third and brightest of which coincides in time with the reported optical maximum. The east-west size of the burst during this maximum is $12''$; assuming the same north-south size we obtain a peak brightness temperature of 10^7 K. This particular peak is about 6% left circularly polarised. The other two peaks have lower brightness temperatures but are more strongly left circularly polarised; the polarisation is of the same sense as that of the associated active region. The position of the maximum emission changes with time, which cannot be due to the change of baseline because this change is very small, indicating that the position of the burst changes during its lifetime.

We thank the Program Committee of WSRT for time on the telescope, Messrs Bregman and Hin for much assistance during the observations and Dr H. Van Someren Greve for help during the initial period of data reduction. We also thank Dr J. van Nieuwkoop and members of WSRT technical group for assistance before the observations, Dr P. Lantos for participating in the observations, and the Leiden and Westerbork observatories for their warm hospitality during our stay. This work was supported by grants from NASA and the NSF. Computer time was supported in part by a NASA grant to the Computer Science Center of the University of Maryland. The Westerbork Radio Observatory is operated by the Netherlands Foundation for Radio Astronomy with the financial support of the Netherlands Organisation for the Advancement of Pure Research (ZWO).

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Special relativity and very long baseline interferometers

WHEN baseline lengths approach the diameter of the Earth, relativistic effects become important, especially in geodetic applications. Failure to take account of these effects results in errors that are equivalent to clock errors of the order of a few parts in 10^{12} .

One method of making the correction has been to use a geocentric coordinate system for the purpose of working out the classical Doppler shift and then to multiply this by a time-dilatation term involving the difference in axial distances between the two receivers. This procedure leads to an erroneous result¹.

The correct approach involves the use of two coordinate systems. One is the source frame of reference and the other is the frame of reference of one of the receivers. To avoid repetition, a table of symbols with their definitions is given in Table 1. The symbols (U) and (O) in the right hand column denote unobservable and observable quantities, respectively. The object of the calculations presented here is to recast an equation involving unobservables into one containing only observables.

Table 1 Definition of symbols

Symbol	Definition
$\mathbf{v}_1$	(U)* Velocity of receiver 1 relative to source (Source frame)
$\mathbf{v}_2$	(U) Velocity of receiver 2 relative to source (Source frame)
$\mathbf{l}$	(U) Unit vector in direction of Earth as seen from source (Source frame)
$\mathbf{l}_1'$	(O)* Unit vector in direction of the source as seen from receiver 1 (Receiver 1 frame)
$\mathbf{l}_2'$	(O) Unit vector in direction of the source as seen from receiver 2 (Receiver 2 frame)
α_1	(U) Angle between $\mathbf{v}_1$ and $\mathbf{l}$ (Source frame)
α_2	(U) Angle between $\mathbf{v}_2$ and $\mathbf{l}$ (Source frame)
α_1'	(U) Angle between $\mathbf{v}_1$ and $\mathbf{l}_1'$ (Receiver 1 frame)
$\mathbf{V}$	(O) Velocity of receiver 2 relative to receiver 1 (Receiver 1 frame)
ϕ_1'	(O) Angle between $\mathbf{l}'$ and $\mathbf{V}$ (Receiver 1 frame)
ϕ_2'	(O) Angle between $\mathbf{l}_2'$ and $\mathbf{V}$ (Receiver 2 frame)
ν	(U) Frequency emitted by source
ν_1'	(O) Frequency received at receiver 1
ν_2'	(O) Frequency received at receiver 2
c	Velocity of light

*U, Unobservable and O, observable quantities.

Except for $\mathbf{V}$, the velocity of receiver 2 relative to receiver 1, unprimed quantities are in the source frame of reference, and thus unobservable, and primed quantities are in the frame of reference of receiver 1. The receivers need not be Earth-bound, but the source and receiver frames are treated as inertial systems.

Following Pauli², the source coordinate axes are arranged so that the x axis is in the direction of $\mathbf{v}_1$. The y axis is in the plane of $\mathbf{v}_1$ and $\mathbf{l}$ and the z axis is perpendicular to x and y . The coordinate axes of receiver 1 are parallel to the source axes, but the two systems are in uniform motion relative to one another. The basic equations to be considered are:

$$\nu_1' = \nu \frac{1 - (\mathbf{v}_1/c) \cos \alpha_1}{\sqrt{1 - (\mathbf{v}_1^2/c^2)}} \quad (1)$$

and

$$\nu_2' = \nu \frac{1 - (\mathbf{v}_2/c) \cos \alpha_2}{\sqrt{1 - (\mathbf{v}_2^2/c^2)}} \quad (2)$$

These are the relativistic Doppler equations applied to the two receivers. Equation (2) can, however, be recast. The vector $\mathbf{v}_2$ is the sum of $\mathbf{v}_1$ and $\mathbf{V}$; $\mathbf{v}_1$ is, however, in the source frame of reference and $\mathbf{V}$ is in the frame of reference of receiver 1 so that it is necessary to resort to the relativistic summation of velocities. The cartesian components of $\mathbf{v}_2$ are, therefore:

$$\left. \begin{aligned} v_{2x} &= \frac{V_x + v_1}{1 + (V_x v_1/c^2)} \\ v_{2y} &= \frac{\sqrt{1 - (\mathbf{v}_1^2/c^2)}}{1 + (V_x v_1/c^2)} V_y \\ v_{2z} &= \frac{\sqrt{1 - (\mathbf{v}_1^2/c^2)}}{1 + (V_x v_1/c^2)} V_z \end{aligned} \right\} \quad (3)$$

α_2 is the angle between $\mathbf{v}_2$, whose direction cosines are v_{2x}/v_2 , v_{2y}/v_2 , v_{2z}/v_2 , and $\mathbf{l}$, whose direction cosines are $\cos \alpha_1$, $\sin \alpha_1$, and 0. Therefore:

$$v_2 \cos \alpha_2 = v_{2x} \cos \alpha_1 + v_{2y} \sin \alpha_1 \quad (4)$$

The denominator of equation (2) is $\sqrt{1 - (\mathbf{v}_2^2/c^2)}$, where $\mathbf{v}_2^2 = v_{2x}^2 + v_{2y}^2 + v_{2z}^2$.

Using equation 3:

$$\sqrt{1 - (\mathbf{v}_2^2/c^2)} = \frac{1}{1 + (V_x v_1/c^2)} \sqrt{\{1 - (V^2/c^2)\}[1 - (\mathbf{v}_1^2/c^2)]} \quad (5)$$

Using equations (1), (3), (4), and (5) in (2):

$$\nu_2' = \frac{\nu_1'}{\sqrt{1 - (V^2/c^2)}} \times \left[1 - \frac{\{(V_x/c)(\cos \alpha_1 - (v_1/c)) + (V_y/c)\sqrt{1 - (\mathbf{v}_1^2/c^2)}\} \sin \alpha_1}{1 - (v_1/c) \cos \alpha_1} \right] \quad (6)$$

From Pauli²:

$$\frac{\cos \alpha_1 - (v_1/c)}{1 - (v_1/c) \cos \alpha_1} = \cos \alpha_1'$$

and

$$\frac{\sqrt{1 - (\mathbf{v}_1^2/c^2)} \sin \alpha_1}{1 - (v_1/c) \cos \alpha_1} = \sin \alpha_1'$$

Therefore:

$$\nu_2' = \frac{\nu_1'}{\sqrt{1 - (V^2/c^2)}} \left[1 - \frac{V}{c} \left(\frac{V_x}{V} \cos \alpha_1' + \frac{V_y}{V} \sin \alpha_1' \right) \right] \quad (7)$$

$(V_x/V) \cos \alpha_1' + (V_y/V) \sin \alpha_1'$ is $\mathbf{V} \cdot \mathbf{l}'$. Therefore:

$$\nu_2' = \frac{\nu_1'}{\sqrt{1 - (V^2/c^2)}} \left[1 - \frac{V}{c} \cos \phi_1' \right] \quad (8)$$

Equation (8) is the required equation. It expresses the frequency received at one receiver in terms of the frequency received at the other. It contains only observables. ϕ_1' is the angle between the source direction measured from receiver 1, and the velocity of receiver 2 relative to receiver 1. The term

$(V/c) \cos \phi_1'$ is similar to the classical Doppler term, but the angle ϕ_1' involves the source direction as seen from receiver 1 and so includes diurnal aberration. If a geocentric coordinate system had been chosen, the Doppler term could not have included diurnal aberration. $1/\sqrt{1-(V^2/c^2)}$ is a time-dilatation factor, and the right hand side of equation (8) is the product of a time-dilatation and a Doppler shift factor; all of the terms must be defined correctly.

It makes no difference which receiver is labelled number 1, and, therefore, equation (8) can equally well be written

$$v_1' = \frac{v_2'}{\sqrt{1-(V^2/c^2)}} \left[1 - \frac{V}{c} \cos \phi_2' \right] \quad (9)$$

From equations (8) and (9):

$$\cos \phi_2' = - \frac{(\cos \phi_1' - (V/c))}{1 - (V/c) \cos \phi_1'} \quad (10)$$

Equations (8) and (10) have the same form as equations (15) and (16) of Pauli². These are the relativistic Doppler equation and the equation for transforming angles from the source frame to the receiver frame of reference. In the simple Doppler case involving one source and one receiver, however, the velocity involved is the relative velocity between source and receiver, whereas in my equations (8) and (10), the velocity involved is the velocity of one receiver relative to the other one. The velocity of the source relative to the receivers, which is unobservable by means of an interferometer, is not involved.

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Received March 7; accepted July 8, 1974.

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Mode of propagation of penumbral waves

THE solar atmosphere provides an interesting possibility for the study of low frequency waves both in magnetic and non-magnetic regions, and the most spectacular result (see ref. 1) has been the discovery of waves propagating outwards over sunspot penumbrae with periods of 180–240 s, horizontal wavelengths of 2,350–3,800 km, and horizontal phase velocities of 12–25 km s⁻¹. The theoretical interpretation of these waves has aroused considerable interest and, in particular, a number of authors have attempted to identify the precise mode of wave propagation involved. The modes proposed include Alfvén waves¹, acoustic waves², Lamb waves³, and magnetogravity waves of the 'plus' type vertically trapped at photospheric levels⁴. In the face of so many possibilities, further progress will probably depend on a more adequate observational knowledge of the actual propagation characteristics of the penumbral waves. Of special importance would be a determination of the direction of the particle velocity with respect to the wave propagation vector.

I have therefore explored the conditions of observability as a function of heliocentric angle for purely longitudinal (that is, particle velocity parallel to the propagation vector) and purely transverse (that is, normal to the propagation vector) waves and I present here observations that tend to support a predominantly longitudinal mode of wave propagation.

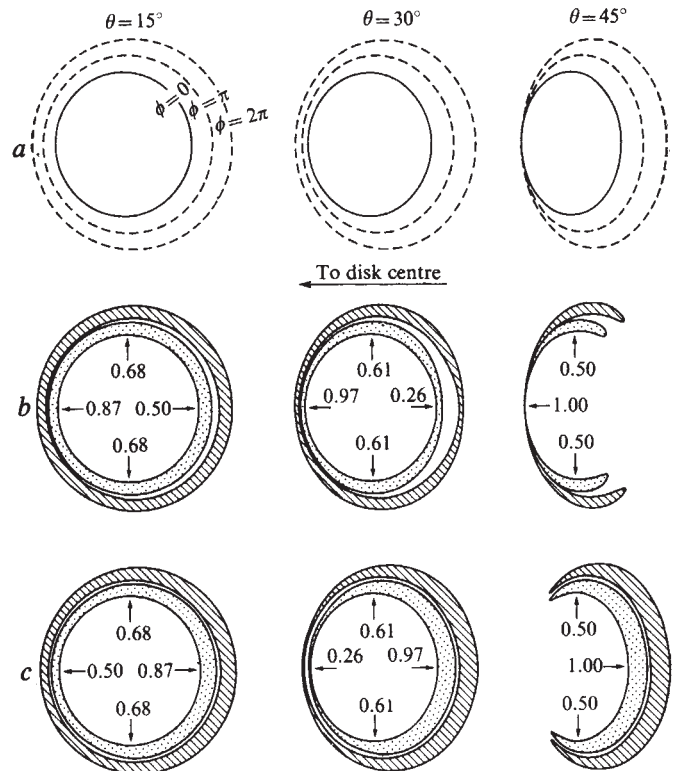


Fig. 1 *a*, Loci of positions with phase $\phi = 0, \pi$, and 2π in a hypothetical wave pattern (see text) for different values of the heliocentric angle θ . The wave is assumed to propagate at an angle $\alpha = \pi/4$ with respect to the vertical. Regions with line-of-sight velocity $|v_{||}| > 0.25v_0$, where v_0 is the amplitude of the wave, are shown for longitudinal waves (*b*), and transverse waves (*c*). The maximum line-of-sight velocity (in units of v_0) is given for certain position angles. Shaded and hatched regions refer to receding and approaching velocities, respectively.

Consider a hypothetical, progressive wave symmetrical with respect to the spot axis, such that at a fixed instant of time the horizontal distance from the centre of the spot is equal to r for positions in the wave with phase $\phi = 0$ and $r + \lambda \sin \alpha$ for $\phi = 2\pi$. Here, λ is the wavelength of the wave, assumed to propagate at an angle α with respect to the vertical. Projected on to a plane perpendicular to the line of sight, the loci of positions in the wave with $\phi = 0$ and 2π represent ellipses. The $\phi = 2\pi$ ellipse is displaced by an amount $\lambda \cos \alpha \sin \theta$ towards the solar limb with respect to the $\phi = 0$ ellipse. Positions in the wave with $\phi = 0, \pi$, and 2π are shown in Fig. 1*a* for different values of the heliocentric angle θ , assuming $\alpha = \pi/4$, $r = 6,500$ km, and $\lambda \sin \alpha = 3,000$ km. As the wave propagation vector is observed to have a horizontal component, a value of α close to zero cannot account for the observations, but a determination of α is not available at present.

Depending on the type of wave involved, variations in the line intensity may⁵ or may not⁶ occur. The line intensity is not a simple function of the particle velocity in the wave. But provided that the wave amplitude is independent of position angle in the spot, positions with the same phase will also show the same line intensity. The appearance of an 'intensity' wave at different heliocentric angles can, therefore, be visualised from Fig. 1*a*, which shows lines of equal phase.

For a longitudinal wave the particle velocity, v , has the components $v \sin \alpha$ and $v \cos \alpha$ in the horizontal and the vertical directions, respectively, whereas for a transverse wave the corresponding components are $-v \cos \alpha$ and $v \sin \alpha$. Assuming that the velocity amplitude v_0 is constant across the region $\phi = 0-2\pi$, we have calculated the line-of-sight velocity $v_{||}$ of the particles in the wave using the equations given by Maltby⁷. The results are given in Fig. 1*b* and *c* which shows the regions

where $|v_{||}|$ exceeds $0.25v_0$ for longitudinal and transverse waves, respectively.

The direction of particle motion in the waves can be studied by comparing observations of spots situated close to the limb with those of spots near the disk centre. Observations of sunspots at large heliocentric angles are, however, hampered because of foreshortening. On the other hand, Fig. 1b and c indicates that significant differences in the observability of longitudinal and transverse waves still exist for spots situated at intermediate heliocentric angles. The observability of the waves evidently depends on the direction of wave propagation (that is, on the value of α). If $\pi/4 < \alpha < \pi/2$, transverse waves are observable more easily than longitudinal waves in spots situated near the disk centre.

We have compared hypothetical wave patterns with actual wave patterns observed in spots photographed at the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Solar Observatory, Culgoora, between 1971 and 1974. No asymmetry was detected in six spots situated relatively close to the centre of the solar disk ($\theta < 23^\circ$). For the spots observed on November 3, 1971 (ref. 1: Fig. 1) and July 3, 1973 (ref. 8: Figs 1–4) penumbral waves are practically absent on the limb side of the spot, but prominent in other places; these spots have heliocentric angles of 35° and 34° , respectively. The observed asymmetries therefore tend to support an interpretation in terms of a predominantly longitudinal mode of wave propagation. The available data are, however, very limited and more observations will be required before any firm conclusion can be reached.

I thank Dr R. G. Giovanelli for allowing me to inspect his sunspot films and E. J. Tappere and N. Bryant for observational assistance.

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Received May 2; accepted August 18, 1975

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Basal complex of Fuerteventura (Canary Islands) is an oceanic intrusive complex with rift-system affinities

THE exposed portions of the Canary Islands largely comprise Cainozoic volcanic rocks erupted sub-aerially in successions essentially specific to each island, though with somewhat complex interrelationships¹. On three islands, Fuerteventura, La Palma, and Gomera these rocks rest unconformably on older formations which are similar enough to suggest that a common basement may exist. The largest area of the basement complex rocks is the Betancuria Massif^{2–4}, Fuerteventura. Gastes⁵ has suggested that similarities exist between the Betancuria Massif and other ophiolite complexes (such as the Troodos Massif of Cyprus^{6,7}) generally held to be representative of oceanic crust formed at certain constructive plate margins.

The most obvious feature of the complex is the striking dyke swarm in which the dyke intensity sometimes reaches 100%. The oldest rocks seen at the present erosion level form the host rock to the dyke swarm and comprise basic and alkaline rocks of plutonic aspect together with marine sedimentary rocks intercalated with submarine volcanics (Table 1a). The sediments comprise laminated shales and

Table 1 Revised succession* from the Betancuria Massif

	Unconformity
Basal Complex	d Late syenitic and trachytic ring complexes Alkali gabbros sometimes related to the ring complexes
	c Basic and ultrabasic intrusions, with associated dykes
	b Main phase dyke swarm
	a Basic and alkaline rocks of plutonic aspect Mesozoic and Tertiary sediments with submarine volcanics

*Date from a reinvestigation conducted during 1974, and recent work by the Instituto Lucas Mallada.

siltstones, turbiditic sandstones and thin tuffs and limestones; Cretaceous foraminifera have also been found⁷. Weak regional metamorphism and metasomatism have affected all the strata; in addition, the sediments close to the ultrabasic and basic intrusions (Table 1c) have been thermally metamorphosed. Deformation has imposed a regional cleavage and much of the observed sequence is inverted by large scale, WNW–ESE trending folds. According to Rothe⁷ these rocks are similar to rocks from the African continent and from Maio in the Cape Verde Islands. At the top of the sedimentary sequence interbedded hyaloclastites and pillow lavas appear, leading up into a thick series of submarine volcanics in which both basaltic and trachytic types are represented. The bedded volcanic rocks are traversed by synchronous sheet intrusions of similar petrography to the pillow lavas.

Because of the subsequent intrusion of a dyke swarm it is difficult to trace stratigraphic continuity. It seems, however, that tuffs become much more common passing upwards: calcareous tuffs appear with thin limestones which become more frequent until thick fragmental limestones containing abundant shell debris are seen. Fossils of Miocene to Eocene age have been recorded from this horizon⁸. It seems that these sediments are of shallow water origin, and according to earlier work² the sequence continues upwards into subaerial tuffs and agglomerates. In the reinvestigated area the succession is terminated by an unconformity above which the Basalt Series was erupted.

In the south-western part of the Betancuria Massif, the host rocks to the dyke swarm comprise basic and alkaline rock of plutonic aspect. The relationship between these rocks and the Mesozoic bedded rocks is not clear as the contact is obscured by the younger dyke swarm. Intruding both the plutonic and the bedded rocks are abundant veins and occasional small intrusive bodies of syenite and small carbonatite dykes which occupy a zone marginal to the plutonic rocks and which do not extend far into the sediments and submarine volcanics.

All of those rocks are traversed by a swarm of dominantly basaltic and ankaramitic dykes with a general NNE–SSW trend (Table 1b). The swarm contains dykes of several ages, but the majority belong to a major phase of permissive emplacement which involved no deformation of the host rock other than distension of the crust. Throughout this phase of emplacement later dykes injected earlier ones and, consequently, individual dykes may be dismembered, with the fragments widely separated. Both the host rocks and dykes of this phase show the imprint of a greenschist facies metamorphism and, as in the oceanic greenstones, are typically devoid of a pervasive foliation⁹. The recognition of thermal aureoles around basic and ultrabasic bodies

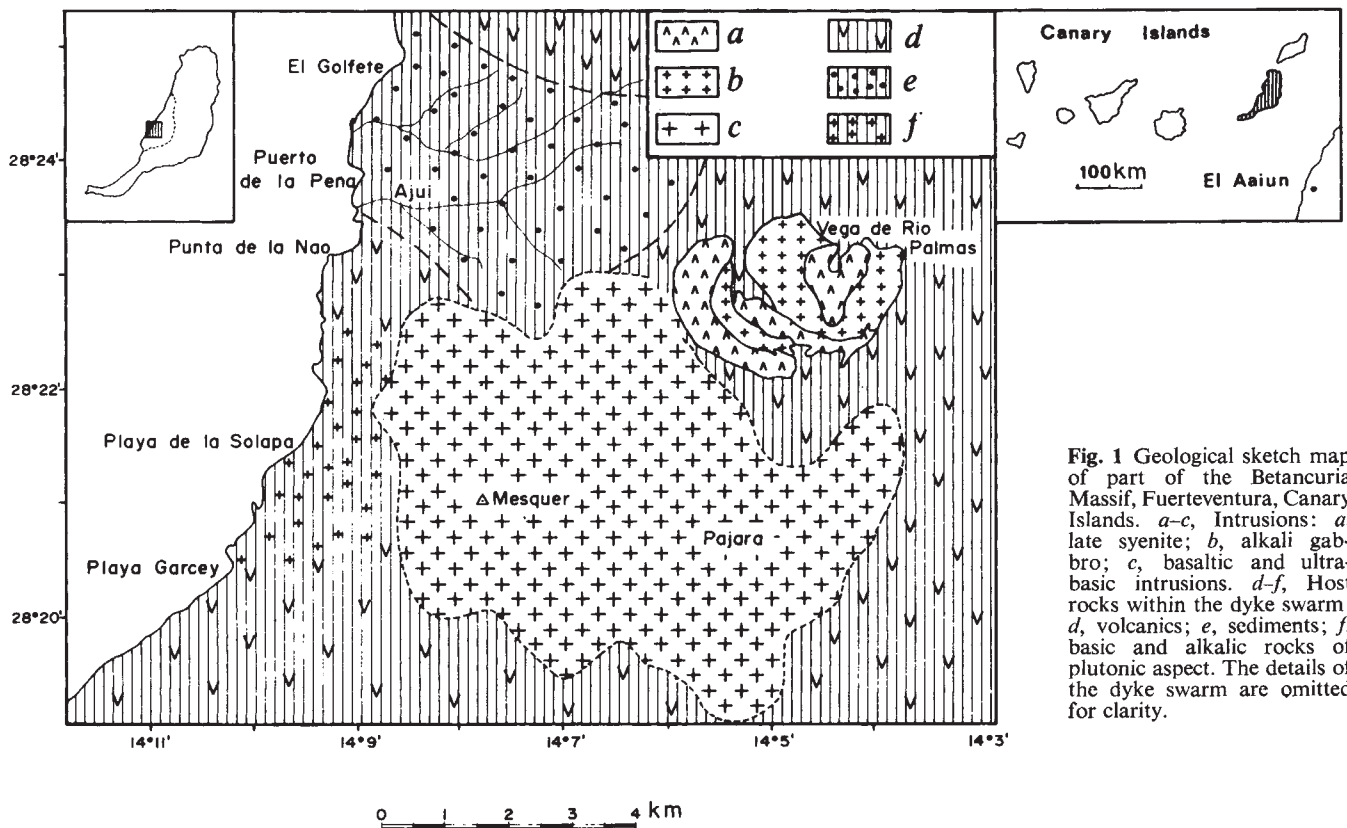


Fig. 1 Geological sketch map of part of the Betancuria Massif, Fuerteventura, Canary Islands. *a-c*, Intrusions: *a*, late syenite; *b*, alkali gabbro; *c*, basaltic and ultrabasic intrusions. *d-f*, Host rocks within the dyke swarm: *d*, volcanics; *e*, sediments; *f*, basic and alkalic rocks of plutonic aspect. The details of the dyke swarm are omitted for clarity.

(Table 1c) which intrude the earlier parts of the complex has made possible the distinction of at least two periods of dyke intrusion in the main phase. The proportion of dykes to host rock increases markedly towards these younger intrusives, implying that during emplacement basaltic magmas from the intruding body was fed actively into fractures within the overlying crust and that it was into that heavily dyked host that the pluton was finally emplaced. The older dykes (whose parent pluton is not exposed at the present erosion level) and those associated with the more recent activity are petrologically similar, are affected by the same greenschist facies metamorphism, and strike in the same direction. The field evidence thus suggests repeated plutonic and hypabyssal intrusion in the same environments within a single extensional stress field.

Subsequent to the relaxation of this stress field, sheet intrusions associated with still later syenitic and trachytic ring complexes were emplaced into the dyke swarm. In addition, younger, zeolite facies basaltic dykes of unknown affinities also occur.

Most of the later dykes follow the same trend as the earlier ones where the dyke intensity approaches 100%, though the concordance is much less noticeable away from this zone. Apparently, the initial trend was dictated by regional tensional stresses, but subsequent emplacement was controlled more by the grain imposed on the crust by the dyke swarm. Some of the ultrabasic and gabbroic intrusions are parallel to the orientation of the dyke swarm; others have been emplaced by passive stoping, sometimes associated with ring fracturing. The latter are succeeded by syenite and trachyte intrusions which form large partial ring dykes and sheet intrusions such as those at Vega de Rio Palmas.

Although the individual components of the Basal Complex, are similar to those forming ophiolites^{4,10}, the relationships which define an ophiolitic terrain¹⁰ are not seen, and it is, therefore, uncertain whether the Basal Complex represents a fragment of normal oceanic crust. There can be little doubt that the bedded rocks were erupted and de-

posited on an ocean floor; but the problem remains: was this part of a marginal basin underlain by continental crust or was it part of the new Atlantic crust? The bedded rocks range in age from the Upper Cretaceous⁷ to the Mid-Tertiary⁸, though that range may be more extensive—Rothe⁷, for example, has suggested that the older sediments may be Jurassic, and that the Mesozoic rocks at least have been involved in Alpine-style tectonics, and are, therefore, presumably related to the Atlas tectonism on the African continent. Further evidence that the sediments form part of a continental margin basin is provided by seismic profiles from the area between Fuerteventura and the mainland, which indicate the presence of some 10 km of sediment¹¹. There is, however, a divergence of opinion as to the nature of the crust beneath these sediments^{2,11-14}. Further, although seismic data confirm the existence of an important fracture west of Fuerteventura^{13,14} there seems to be little in the available petrological and geochemical data to suggest that the islands in the west and east of the archipelago are emplaced over significantly different crust. Data from the basal complexes do not indicate much difference in the early stages, and studies^{15,16} of Cainozoic magmas reveal inter-island relationships which are complex, and do not suggest any particular relationship between geochemistry and distance from the African continent.

Radiometric data suggest that the majority of the Fuerteventura Basal Complex intrusives were emplaced in mid-Tertiary times. Ages from dykes of the main phase range from 32 Myr to 46 Myr; the age of the older basic and alkalic rocks of plutonic aspect is not yet known, and the only pluton which has so far been dated (20 Myr) is one of the youngest: the outer ring syenite of the Vega de Rio Palmas Complex (ref. 17, and D. Rex and I. G. Gass, personal communication). Basal Complex activity must have terminated shortly after 20 Myr BP, as the beginning of subaerial volcanicity has been dated at >16.5 Myr (ref. 17).

The tensional conditions which permitted the intrusive phase on Fuerteventura may well represent mid-Tertiary

rifting and may indicate the existence of an embryonic spreading axis which aborted after a relatively short time, a view already postulated by Gastesi¹. The extensional stress field had probably relaxed by the time of emplacement of the syenite ring complexes approximately 20 Myr BP. The variations in trend of the Basal Complex dyke swarms on La Palma, Gomera and Fuerteventura may indicate several 'spreading' attempts but until more precise chronological correlation is possible, that remains speculative. The case for a propagating fracture model to explain the continuing volcanicity of the Canary Islands has been propounded¹, and the proposed model for mid-Tertiary rifting does not conflict with that idea.

This work is a preliminary report of a joint research project led by Professor J. M. Fuster of Madrid and Professor I. G. Gass of the Open University, UK. The British members of the project acknowledge financial support from the National Environment Research Council. We also thank A. Hernandez Pacheco, F. Anguita, F. Hernan and J. M. Navarro, I. G. Gass, M. J. Le Bas, P. Gastesi and A. Cendrero for field assistance and discussions.

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Received July 7; accepted August 29, 1975.

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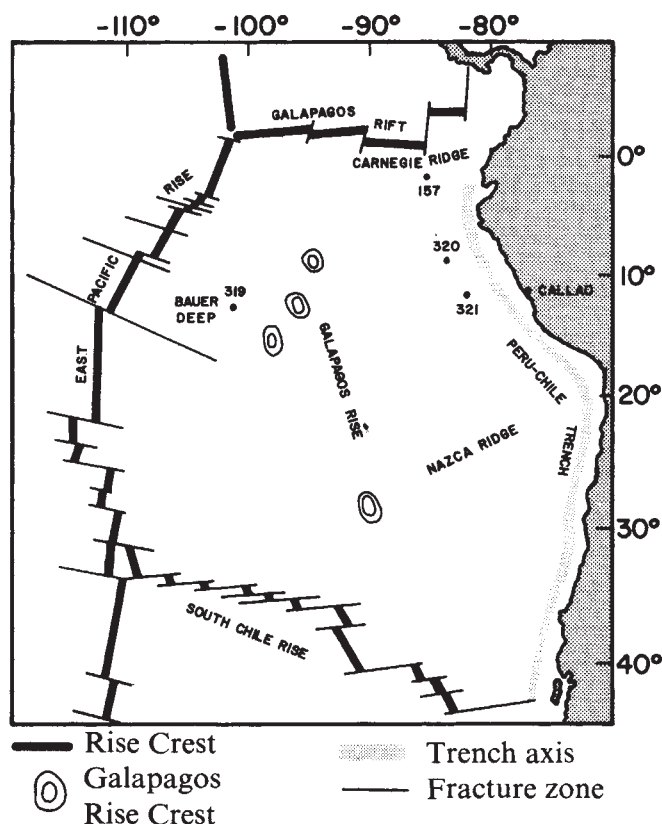


Fig. 1 The Nazca Plate showing DSDP sites 319, 320, 321 and 157.

that the determination of the ages of sites 319 and 320 with respect to the magnetic reversal time scale will be precluded. Table 1 shows the basement ages for the sites, estimated from the overlying sediments.

The total penetration of basement rocks at three sites was 105 m, of which only 15 m were recovered for study. A total of 35 basalt and 32 sediment samples (vertically oriented) was taken from the leg 34 drill sites. In addition, we have studied four samples from site 157 of the DSDP leg 16, also on the Nazca Plate.

Recovery of coarse grained massive basalts at several sites, in addition to pillow sequences, justifies the previous suggestion⁴ that dredged material from the upper surface of oceanic layer 2 is unrepresentative of the layer as a whole. Coring in the massive flow units produced good recovery at sites 319 and 321, and pillow material resulted in poor recovery at all sites. Table 1 shows the average natural remanent magnetisation (NRM) intensity for each site, weighted for the differential recovery of fine and coarse material. Pillow material was significantly less intensely magnetised than the coarse grained flow interiors, and this is shown by comparison of the average magnetisation at sites 321 and 157, which were dominated by

Magnetic results from basalts and sediments from the Nazca Plate

THE drilling sites for leg 34 of the Deep Sea Drilling Project (DSDP) were in the Nazca Plate (Fig. 1), which was formed by the spreading from two ridges, the fossil Galapagos Rise and the currently active East Pacific Rise. Spreading half rates on the East Pacific Rise vary from 8 to 10 cm⁻¹ (refs 1-3) and are the highest on any presently active ridge. Fast spreading is associated with subducted basement topography, a situation that enables structural provinces within the Nazca Plate to be clearly delineated. The near equatorial latitudes of the sites with poor magnetic anomaly development mean, however,

Table 1 Basement ages and average intensity of undemagnetised natural remanent magnetisation for the basement basalts

Site	Estimated age of sediment directly above basement (Myr)	Site average NRM intensity (e.m.u. cm ⁻³ × 10 ⁻⁴)	
		Averaged over all NRM values	Weighted for differential recovery of fine and coarse grained basalts
157	8	138.0 ± 24.0	—
319	14-15	23.8 ± 5.7	17.4 ± 5.9
320	26-30	13.2 ± 2.9	13.2 ± 7.6
321	39-40	103.0 ± 23.0	96.0 ± 23.0

Table 2 Mean site inclinations and equivalent palaeolatitudes for sediments and underlying basement basalts

Site	319	320	321	157
Site latitude	13°S	9°S	12°S	2°S
Equivalent dipole inclination	25°	18°	23°	4°
Basalt average inclination	+54±4°	-25±9°	-20±2°	-31±4°
Equivalent dipole palaeolatitude (north or south)	35±4°	13±5°	10±1°	17±3°
Sediment average inclination				
(a) Assuming all samples have same polarity	14±7°	8±10°	11±4°	
Equivalent dipole palaeolatitude (north or south)	7±4°	4±6°	6±2°	
(b) Assuming polarity indicated by sign of inclination	16±6°	31±6°	15±3°	
Equivalent dipole palaeolatitude (north or south)	8±3°	17±4°	8±2°	

coarse grained material, and site 320, which was exclusively oxidised pillow basalts. Site 319 is a 60-m basement section, and the remanence is dominated by a 10–20-m section of coarse grained flow material.

For the basalt samples, the ratio of remanent intensity to induced magnetisation, Q , was in all cases greater than 1. The range of Q was large, for massive flows varying from 2 to 28 and for fine grained basalts varying from 2 to 56. It would seem that for these samples, the remanent intensity will be dominant.

The coarse grained basalts of hole 319A had very large, soft components of magnetisation that were directed vertically upwards. This hole was drilled with a magnetic steel drilling assembly, a configuration that is sometimes responsible for a drilling remanent magnetisation. Lowrie and Kent⁶ have shown that for some of the leg 34 coarse grained samples, exposure to a field as small as 4 oersted for only a few hours, a time sufficient to drill 9.5 m of basalt, can generate the observed soft components. The massive flow basalts of site 321, which were drilled with a non-magnetic monel metal drilling assembly, had soft secondary components of magnetisation which were directed downwards. At all of the leg 34 sites, the geomagnetic field, at present, is inclined upwards at a shallow angle. The soft components of NRM measured in the laboratory seem to be some combination of drilling and viscous remanent magnetisation (VRM) acquired on the ocean floor. Lowrie and Kent⁶ have also shown that these coarse grained samples can acquire a VRM component that is comparable in intensity with the stable component in less than 7×10^6 yr. If this is the case, then *in situ* VRM will be the dominant remanence and should mask or distort any stable thermoremanent magnetisation (TRM). In areas of the oceanic crust which are predominately coarse grained basalts, the magnetic anomaly which is evident at the sea surface will be composed of some combination of a viscous remanence, the induced magnetisation (both in the direction of the present field), and the original thermoremanence. In these areas, the magnetic anomalies will tend to be positive, and the variations in the anomaly pattern will be unlikely to correspond to a magnetic reversal time scale.

Table 2 shows the inclinations for the partially demagnetised basalt samples, averaged for each site. The values for sites 320 and 321, the oldest sites, are close to those expected for an axial

centred dipole at the latitude of each site. Sites 157 and 319, however, show significant departures from the inclinations expected at the present-day latitudes of the sites. Interpretation of these inclinations as actual palaeolatitudes would require unusually large plate velocities of 20 cm yr⁻¹ and would also require that the Nazca Plate moved rapidly southward between 26 and 15 Myr ago, and then northward to the present latitude between 15 Myr ago and the present.

A more reasonable explanation is that the basalts taken at sites 157 and 319 were erupted over a time interval that was too short to average over a quasi-period (10^3 yr) of secular variation. A possible, but less likely explanation is that during extrusion, the geomagnetic field was undergoing a polarity transition or some systematic deviation. Tectonic movement of crustal blocks, although present to a small extent, is an unlikely cause of the rotation of the magnetic directions. Chemical remagnetisation due to oxidation can be eliminated since similar directions are indicated for both the oxidised and unoxidised samples. The tight and consistent grouping of the inclinations of site 319 indicates that a short period of extrusion is the likely explanation.

Additional information about the palaeolatitude of the Nazca Plate is available from the sediments which overlie the basement basalts. Since the sedimentation rate in this area has been estimated to be 2.8–4.2 cm every thousand years⁵, each sediment sample with a diameter of 1.9 cm covers about 450–680 yr. At each site, the sediment column directly above the basement was cored over a few tens of metres, a thickness representing about 10^6 yr. When the sampling interval is extended over this longer time period, the sediment inclinations in Table 2 show a southward palaeolatitude change of 3–5° for the Nazca Plate during the past 15 Myr and no latitude change between 15 and 40 Myr BP. This agrees with previous studies^{1–3,6} that the motion of the plate has been largely in an eastward direction.

The study of the palaeomagnetism of oceanic basement rocks can provide information regarding a rough minimum of the amount of time involved in crustal genesis. The presence of several magnetic reversals in a column of basalt flows implies a time of formation of 10^5 – 10^6 yr or greater, whereas a tight grouping of inclinations of a single polarity (that deviate

Table 3 Comparison of rock magnetic parameters for site 319

Type of material	Mean particle size (μm)	Curie temperature T_c (°C)	Median demagnetising field MDF (oersted)	Weak field susceptibility k (e.m.u. cm ⁻³ × 10 ⁻⁴)	Intensity of NRM J_0 (e.m.u. cm ⁻³ × 10 ⁻⁴)	Strong field saturation moment J_s (e.m.u. cm ⁻³)
Oxidised pillows or flow margins (5)*	4.9	340	258	0.86	8.6	0.50
Oxidised coarse grained massive flow interiors (4)	20.0	290	167	1.26	12.0	0.97
Unoxidised coarse grained massive flow interiors (5)	19.0	147	64	8.4	65.0	1.4

Samples were grouped into one of three categories for comparison of parameter variation with low temperature oxidation state and grain size

*Numbers in parentheses are number of samples

significantly from the expected inclination) may imply extrusion within 10^3 yr. The inclination data from site 319 suggest that the basement crust sampled at this site was formed in a relatively short time. Too few oriented samples were recovered from site 320 for any interpretation to be made. The inclinations from site 321 were tightly grouped with the exception of one reversed inclination from a sample at the very top of the basement section. Re-examination of the drill core showed that this section had been inverted during drilling. If this reversal is the result of coring, then the column of basement basalts at this site would also have been generated over a short ($\leq 10^3$ yr) time period. At site 157 the three top samples were normally magnetised and overlie a reversely magnetised sample. Re-examination of the drill core showed no evidence that this reversal was the result of drilling. If this bottom sample represents a true field reversal, then this column of basement basalts is recording a period in which the geomagnetic field is reversing or the time of formation of this thin layer of ocean crust is greater than 10^5 – 10^6 yr.

The basalt samples were divided into three categories, based on the size of the titanomagnetite grains present and the degree of alteration of these grains by low temperature oxidation.

(1) The first category consists of the unoxidised interiors of massive volcanic units characterised by large unoxidised titanomagnetite grains, 20–30 μm in mean size with grains up to 100 μm .

(2) Second, there are samples similar to the first, but now oxidised to titanomaghaemite and physically subdivided by cracks due to alteration.

(3) Lastly, there are fine grain pillow basalts with skeletal grains of titanomaghaemite, 2–5 μm in size. No unoxidised pillow material was recovered.

Division of the basalt samples into these categories makes possible the correlation of the magnetic parameters with low temperature oxidation state and grain size. Data from Site 319, which contained all three types of basalt samples, enable comparisons to be made without a wide variation in age or geographic location. Table 3 shows the magnetic properties for these groups of samples. The unoxidised massive flows are more intensely magnetised than either the oxidised massive flows or the oxidised pillow basalts. Similar to what has been reported from laboratory studies⁷, the natural low temperature oxidation of titanomagnetite to titanomaghaemite causes a decrease in intensity of remanence and saturation moment, and an increase in coercivity and Curie temperature. Weak field susceptibility also decreases with oxidation.

Low temperature oxidation is important in the control of the magnetic properties of submarine basalts, particularly in the fine grained pillow material. Evidence for hydrothermal alteration at moderate temperatures was absent from all four sites on the Nazca Plate. The presence of unoxidised titanomagnetite grains in the massive volcanic units of the 40-Myr-old site 321 and 15-Myr-old site 319 indicates that flow structure can be a more important factor in the alteration history than age alone.

We thank Professor E. E. Larson for a constructive review.

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Evidence for a new aluminium phosphate phase from reaction rate of phosphate with aluminium hydroxide

THE reaction between phosphate and aluminium hydroxide is important in soil fertility and also in the management of systems whereby waste water is disposed directly on to the land. The retention of phosphate by a sandy topsoil during 50 yr of application of raw sewage water cannot be accounted for by adsorption on soil constituents alone¹. It was suggested that precipitation of the phosphate had probably taken place. Later investigations on this soil (Beek, personal communications) indicated the presence of an aluminium phosphate as the main fraction of phosphate retained. We report here results for the production of aluminium phosphate which may have a bearing on waste water management.

Most of the older research on the precipitation of aluminium phosphate involved systems using high phosphate concentrations and/or high temperature^{2–5}. The experiments reported here were done at 20.5 ± 0.1 °C with an initial phosphate concentration of $0.36 \text{ mmol dm}^{-3}$ at pH 5. X-ray amorphous aluminium hydroxide with a specific surface area (B.E.T.) of $226 \text{ m}^2 \text{ g}^{-1}$ was used⁶. The pH was kept constant (± 0.1) by the addition of HCl. From aliquots of the suspensions the phosphate concentration was measured after filtration through a Millipore (0.1 μm) filter.

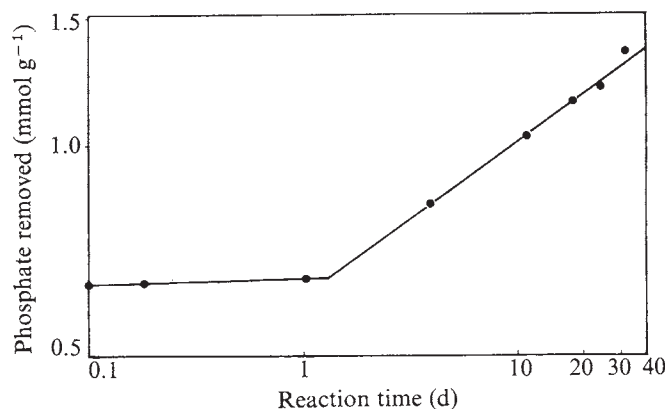


Fig. 1 A semi log plot of the measured phosphate concentration as a function of reaction time for a suspension concentration of $0.2 \text{ g dm}^{-3} \text{ Al}(\text{OH})_3$. For the period 1–40 d, the first order rate constant, $K_{1-40 \text{ d}}$ equals 0.0069 d^{-1} .

The results of the measurements are given in Figs 1 and 2. Figure 1 shows that, for the time period from ~ 1 to 40 d, the rate can be described by first order kinetics.

$$\ln \frac{c_0'}{c} = Kt \quad (1)$$

where $c_0' = c$ at about 1 d of shaking, K = rate constant d^{-1} , c = phosphate concentration. Similar observations have been reported⁷ for the reaction of phosphate with $\alpha \text{ Al}_2\text{O}_3$ and with kaolinite.

The value of K depends on the amount of specific surface area per litre and on the charge of the surface which is dependent on pH. Another way to express the measured data is in terms of the amount of phosphate removed from the solution per g $\text{Al}(\text{OH})_3$. This is shown in Fig. 2 using a double log plot.

Figure 2 shows two different straight lines. The first line (up to about 1 d) represents the fast adsorption on the surface of the

$\text{Al}(\text{OH})_3$, a short term reaction which has been shown previously⁸. After 1 d the reaction rate increases markedly compared with the first process, a phenomenon which remains hidden in Fig. 1. Such an increase in reaction rate could conceivably be caused by the initiation of the growth of a crystalline aluminium phosphate. This supposition was tested using electron microscopy, electron diffraction and X-ray diffraction. These techniques were applied after a reaction time of 62 d. Crystalline particles of a platy structure were visible, some of them

From the evidence given here it is not unlikely that the growth of an aluminium phosphate phase is the rate determining step after 1 d in this system. Similar experiments were done with $\alpha\text{-Al}_2\text{O}_3$ and with solutions containing different ions at pH 5 and 6. It is interesting to note that the intersection point of the two straight lines in Fig. 2 occurs at a reaction time of 4 d for the case of $\alpha\text{-Al}_2\text{O}_3$. The results of these experiments which are comparable with the ones given here will be published elsewhere.

Table 1 *d* Spacings of the aluminium phosphate investigated compared with the tabulated values of sterrettite, meta-variscite and variscite

X-ray diffraction	Electron diffraction	Sterrettite*	Tabulated values of			
			Meta-variscite*		Variscite*	
		6.94	6.37 (4)	2.16	5.365 (1)	1.952
		5.25	4.77	2.11	4.82	1.903
	5.26	4.88 (1)	4.575 (3)	2.07	4.257 (1)	1.852
4.90 (1)		4.51 (2)	4.43	2.01	3.887	1.809
4.51 (2)	4.55	3.79 (4)	4.25 (2)	1.961	3.625	1.779
3.75		2.90 (3)	4.04	1.933	3.366	1.752
		2.76 (5)	3.52	1.865	3.204	1.715
	2.63	2.66	3.37	1.824	3.039 (1)	1.656
2.62		2.44	3.25	1.793	2.957	1.640
		2.33	3.12	1.771	2.853	1.604
	2.25	2.24	3.02	1.743	2.63	1.588
		2.07 (6)	2.90	1.721	2.568	1.574
		1.87	2.79	1.670	2.48	1.557
		1.77	2.71 (1)	1.646	2.39	1.519
	1.72	1.71 (7)	2.65	1.630	2.33	1.496
		1.61	2.59	1.612	2.278	1.438
	1.52	1.54	2.50	1.591	2.136	
		1.49	2.41	1.560	2.081	
		1.37	2.30	1.539	2.047	
		1.35	2.21		2.014	
	1.31	1.31		plus 15 lines to 1.258		

* Sterrettite (ASTM 2.0177); meta-variscite (ASTM 15-311); variscite (ASTM 15-281). Numbers in parentheses refer to intensities.

exhibiting an hexagonal shape, the size ranging from 0.5 to 4 μm .

Crystals with the correct thickness all showed electron diffraction patterns of which the *d* spacings were measured using gold pattern as reference. All the crystals examined gave the same diffraction pattern. Table 1 shows these *d* spacings along with those obtained by X-ray diffraction and those of the minerals sterrettite, variscite and meta-variscite.

There is good agreement between the measured values and those of sterrettite. The formation of this compound from solutions containing aluminium and phosphate at pH 5.5 has been reported before⁵.

We thank Mr S. Henstra, J. D. J. van Doesburg, and A. B. de Vries, for their work on the electron microscopy, X-ray diffraction and electron diffraction, respectively. This research was supported in part by a grant from the Commission on Manure and Odour Problems in Animal Husbandry.

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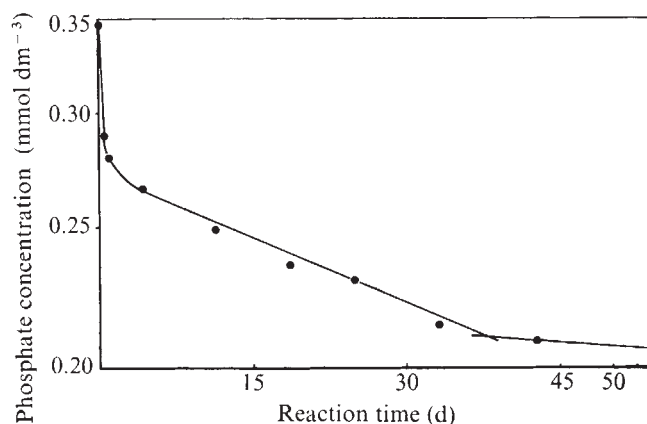
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Fig. 2 A double log plot of the amount of phosphate removed as a function of reaction time. Expressing the lines shown as, $\log X = \log K' + \phi \log t$, with X = phosphate removed (mmol g^{-1}), K' phosphate removed at $t = 1$ d, (ϕ and K' are constants).

$\log K' = -0.19$; $\phi = 0.010$; $t < 1.1$ d.
 $\log K' = -0.20$; $\phi = 0.230$; $t > 1.1$ d.



Excess of atmospheric neon in pumice from the Islands of Lipari

LORD RAYLEIGH¹ reported an excess of neon (over argon) in pumice from the Lipari Islands. Dymond and Hogan² have reported excesses of neon in mid-oceanic tholeiitic basalts and have suggested that they indicate primordial neon. As the search for primordial gases in terrestrial rocks has an important bearing on our understanding of the formation and thermal history of the Earth, we decided to look at the isotopic composition of the neon in pumice from the Lipari Islands. We

Table 1 Helium, neon and argon contents in the Lipari pumice*

Sample	Treatment	^4He ($\times 10^8 \text{ cm}^3 \text{ g}^{-1}$ at STP)	^{20}Ne ($\times 10^8 \text{ cm}^3 \text{ g}^{-1}$ at STP)	$^{20}\text{Ne}/^{22}\text{Ne}$	$^{22}\text{Ne}/^{21}\text{Ne}$	^{36}Ar ($\times 10^8 \text{ cm}^3 \text{ g}^{-1}$ at STP)	$^{36}\text{Ar}/^{38}\text{Ar}$	$^{40}\text{Ar}/^{36}\text{Ar}$	$^{20}\text{Ne}/^{36}\text{Ar}$
A (9.02 g)	Crushing in vacuum	1.22 ± 0.33	9.0 ± 1.1	9.64 ± 0.24	35.4 ± 1.1	0.57 ± 0.06	5.35 ± 0.04	292.6 ± 3.0	15.8 ± 2.0
B (3.22 g)	1st step Crushing in vacuum	19.8 ± 0.31	130 ± 4			20.2 ± 2.0	5.22 ± 0.20	292.5 ± 3.2	6.43 ± 0.90
	2nd step Heating to $\sim 400^\circ\text{C}$	1.6	64.5 ± 6.5	9.80 ± 0.06	35.2 ± 0.1	7.0 ± 0.8	5.30 ± 0.04	293.1 ± 5.4	9.21 ± 0.70
	3rd step Heating to $\sim 1,000^\circ\text{C}$	2.73 ± 0.93	14.0 ± 1.6	9.53 ± 0.29	35.7 ± 1.0	9.6 ± 1.0	5.29 ± 0.04	280.2 ± 1.9	1.46 ± 0.22
	Total (1 + 2 + 3)	22.5 ± 3.6	208 ± 35	9.80 ± 0.06	35.2 ± 0.1	36.6 ± 2.4	5.29 ± 0.04	289.5 ± 3.0	5.65 ± 0.51
C (0.452 g)	Heating to $\sim 1,000^\circ\text{C}$		517 ± 86	9.76 ± 0.14	34.9 ± 0.4	82.9 ± 8.8	5.30 ± 0.06	293.0 ± 2.6	6.23 ± 0.61
Atmosphere				9.80	3.45		5.35	295.5	0.52

*The quoted errors correspond to a 95% confidence limit. They do not include the error of the standard. An error of about 5% has to be added to the given concentrations to obtain the absolute error.

collected fresh specimens from the largest quarry on the islands and extracted the included gases by crushing in a vacuum line and by stepwise heating. The five noble gases were measured in a mass spectrometer.

Large differences were observed in the noble gas contents in three samples (Table 1) A, B and C, indicating heterogeneity in the noble gas distribution, a feature that also seems to be common in basalts. In all of the samples significant excesses of neon were observed, the $^{20}\text{Ne}/^{36}\text{Ar}$ ratio reaching the value 15.8, as compared with the atmospheric value of 0.52. This excess neon has $^{20}\text{Ne}/^{22}\text{Ne}$ and $^{22}\text{Ne}/^{21}\text{Ne}$ values that are indistinguishable from the atmospheric ratios, in the limit of the given analytical errors (Table 1).

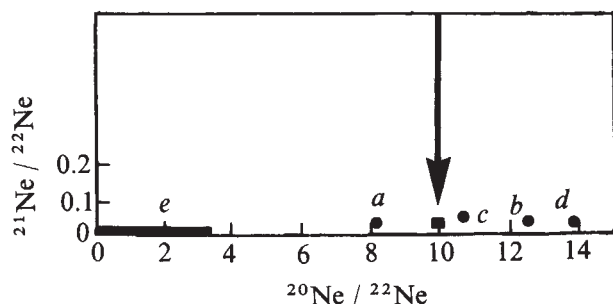


Fig. 1 The isotopic composition of the excess neon in the Lipari pumice compared with known species of primordial neon in extraterrestrial sources (following ref. 3); a, neon A; b, neon B; c, neon C; d, solar wind; e, neon E; arrow, Lipari pumice and atmosphere.

The fact that the same type of neon is observed in crushing and stepwise heating experiments indicates that only one type of neon is present. Further, the observed isotopic composition deviates from any known species of primordial neon in extraterrestrial material (Fig. 1).

These data seem to indicate that the excess neon in pumice from the islands of Lipari is of atmospheric origin. Similar isotopic measurements in the mid-oceanic basalts might clarify

whether they are primordial or atmospheric. The mechanism by which the pumice takes up neon upon its formation needs further study (the fact that the helium is less enriched could be a result of subsequent diffusional losses).

We acknowledge the support of the Israel National Commission for Basic Research.

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Long range transport of toxaphene insecticide in the atmosphere of the western North Atlantic

OVER the past ten years toxaphene has been used in the USA in larger quantities than any other insecticide, with an estimated consumption of 58 million pounds per year (ref. 1). In spite of this widespread use, the composition of toxaphene remains largely undetermined, and recent work indicates that the technical product contains nearly 200 polychlorinated camphenes². Because of the difficulties in analysing such a complex mixture, little is known about the dissipation of toxaphene in the environment. Residues in soils may persist for years, with volatilisation suggested as a major loss mechanism³. Toxaphene has been identified,

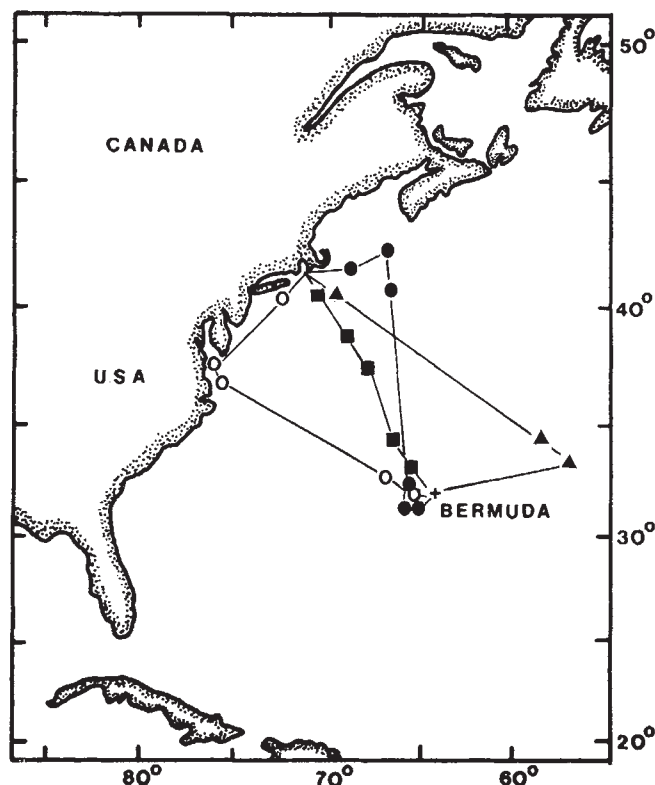


Fig. 1 Atmospheric samples collected from the RV Trident, 1973-74 (see Table 1 for cruise dates). ■, TR-137; ○, TR-145; ●, TR-152; ▲, TR-153.

however, in US air samples in only three locations⁴, all in southern agricultural areas, at levels of 16–2,520 ng m⁻³. The ability of high molecular weight chlorinated hydrocarbons to be transported long distances through the atmosphere has been well established within the past decade. Polychlorinated biphenyls (PCBs)^{5,6}, and the chlorinated pesticides DDT⁸⁻⁹, dieldrin^{7,9}, and chlordane⁶ have been identified in the air over the western North Atlantic. We now report that toxaphene is also carried through the atmosphere at least 1,200 km out to sea.

During 1973–74 we collected 36 air samples from a tower on the south shore of Bermuda and 20 from the bow of the RV Trident on cruises in the western North Atlantic (Fig. 1), and in the spring of 1975 we took six samples at Sapelo Island, Georgia—two on the island and four from the RV Kit Jones, 6–20 km from the coast. The collection system in Bermuda was controlled by wind direction, which enabled us to sample air blowing from a desired sector¹¹. Of the 36 Bermuda samples, 26 were taken only when the wind blew from inside a 90°E–240°SW selected sector (ISS). This air passed over hundreds of kilometres of open ocean and not over the island of Bermuda. Seven samples were collected when the wind blew from outside this sector (OSS) and thus over portions of the island, and the remainder were collected during periods of variable winds.

The samples were taken by drawing 200–3,000 m³ of air through a Gelman A glass fibre filter and then through plugs of polyurethane foam^{6,10}. In most cases we analysed only the foam plugs, since previous work indicated that other chlorinated hydrocarbons are poorly retained by glass fibre filters when large air volumes are sampled. We checked this for a few samples, and found that less than 5% of the total toxaphene was retained by the glass fibre filters, the bulk of the pesticide being found on the foam plugs.

Toxaphene was identified by electron capture gas chromatography on both 1.5% OV-17–1.95% QF-1 and 4%

SE-30–6% QF-1 columns after separation from PCB, DDT, and chlordane by silicic acid column chromatography. The 'toxaphene fraction' from the silicic acid column (3 g, 3.3% water), eluted with dichloromethane after eluting the PCB and DDT–chlordane fractions with petroleum ether, contained the components contributing 60–70% of the total toxaphene electron capture response before separation. Gas chromatograms of the sample extracts and a toxaphene standard contained a number of peaks reminiscent of a PCB pattern (Fig. 2a and b) which were unchanged by treatment of the sample or standard with a mixture of concentrated sulphuric and fuming nitric acids, a procedure which removes residues of DDT, dieldrin, and organophosphate pesticides^{12,13}. The presence of toxaphene was further confirmed by refluxing the sample extracts with alcoholic potassium hydroxide and comparing the resulting gas chromatograms with those of authentic dehydrochlorinated

Table 1 Concentrations of airborne toxaphene

Location*	Dates	Toxaphene† (ng m ⁻³)
Bermuda 32°15'N 64°50'W	Mar.–Dec., 1973	< 0.02–3.3, 1.0 (11)‡
Bermuda 32°15'N 64°50'W	Mar.–Oct., 1974	0.10–1.9, 0.57 (25)‡
Sapelo Island, Georgia 31°15'N 81°05'W	May 20–26, 1975	1.7–5.2, 2.8 (6)‡
Cruise TR-137 33°20'N, 65°15'W 34°40'N, 66°15'W 37°40'N, 68°10'W 38°50'N, 69°15'W 40°30'N, 70°20'W	June 4–9, 1973	0.70 1.2 1.1 0.52 1.6
Cruise TR-145 32°00'N, 65°00'W 32°40'N, 67°20'W 36°40'N, 75°20'W 37°00'N, 76°00'W 37°20'N, 76°10'W 40°30'N, 72°40'W	Oct. 20–31, 1973	0.87 0.57 0.39 0.77 0.77 0.65
Cruise TR-152 31°20'N, 64°50'W 31°30'N, 65°20'W 32°00'N, 65°30'W 40°40'N, 66°30'W 41°30'N, 68°30'W 42°20'N, 66°40'W	May 9–19, 1974	0.16 < 0.1 0.39 < 0.06 < 0.04 0.05
Cruise TR-153 33°40'N, 57°00'W 34°30'N, 58°40'W 40°30'N, 70°00'W	May 25–31, 1974	0.11 0.58 0.04

*Midpoint of the collection track.

†The mean blank corresponded to 0.03 ng m⁻³ for a 1,000-m³ sample.

‡Range, mean and (number of samples).

toxaphene (Fig. 2c and d). Toxaphene was quantitated by comparing the sum of the peak heights in sample and standard chromatograms.

Atmospheric concentrations of toxaphene over the western North Atlantic are reported in Table 1. For several reasons we think that the majority of toxaphene in the air did not originate from Bermuda: (1) the Bermuda Department of Agriculture and Fisheries assured us that toxaphene has not been used on the island for the past 3–4 years; (2) the mean toxaphene concentration of Bermuda samples collected OSS (0.81 ± 0.45 ng m⁻³) (precision reported as s.d.m.) was little different from the mean of those taken when the wind blew ISS (0.72 ± 0.09 ng m⁻³); and (3), comparable levels were measured over the open

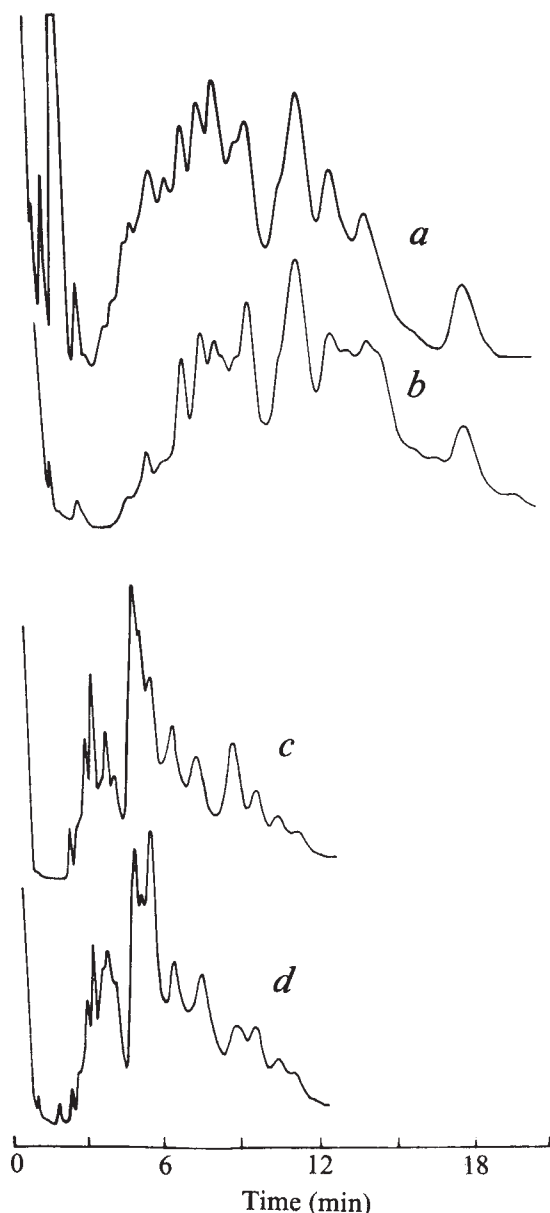


Fig. 2 Gas chromatographic identification of toxaphene in western North Atlantic air, 4% SE-30/6% QF-1 column. *a*, Bermuda air sample; *b*, toxaphene standard, 3 ng; *c*, Bermuda air sample, after dehydrochlorination; *d*, Toxaphene standard, 1.4 ng, after dehydrochlorination.

ocean, indicating that our Bermuda values were unlikely to be the result of local contamination.

Our mean levels of toxaphene in western North Atlantic air are about equal to or twice those of PCBs^{3,6}, and more than ten times higher than those of other pesticides so far reported in the marine atmosphere. These higher residues probably result from both the large scale use of toxaphene and its volatility. The mean toxaphene concentration (ng m⁻³) in our 56 samples from Bermuda and RV Trident was 0.63, as against 0.024 for *p,p'*-DDT. We note that the ratio of these concentrations (26) is close to the ratio of the outdoor evaporation rates of the two pesticides (25) (ref. 3). A possible source of airborne toxaphene is the southern US cotton growing areas, where the bulk of this pesticide has been used in combination with DDT (up to 1973) or methyl parathion¹.

We thank the US Naval Underwater Sound Laboratory, Tudor Hill, Bermuda, for providing a site for the construc-

tion of the air sampling tower, and the University of Georgia Marine Institute, Sapelo Island, Georgia, for providing ship time on the RV Kit Jones. We are grateful to Paul DesLauriers and Ian Fletcher for their help in sample collection.

This work was supported by the Office of the International Decade of Ocean Exploration, under a grant from the National Science Foundation.

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New interglacial site at Sugworth

A PRELIMINARY account is given of a richly fossiliferous interglacial deposit of Middle Pleistocene age at Sugworth, near Oxford, UK.

Excavations for the A34 Abingdon bypass during 1972–73 revealed four fossil channels cut into the Kimmeridge Clay (Figs 1 and 2). The tops of the channels lie at 92 m OD, 40 m above the present level of the River Thames, and seem to represent a former meandering river trending roughly from north to south. In one section near Sugworth Lane (OS ref.: SP 513007; Fig. 2) the channel fill, approximately 180–200 m wide and up to 5 m deep, included organically-rich silts and sands passing laterally into sands and gravels and upwards into about 0.5 m of yellow silty clay. Across the channel fill an unconformable layer, 1–2 m thick, of pebbly sandy clay could be followed along the cutting for 100 m or so beyond the channel. This latter deposit has been mapped by the UK Geological Survey (Sheet 236) as 'Plateau Gravel' and 'Unbedded Drift', the first designation identifying it with the Plateau Drift of the Oxford region which most workers have regarded as wholly or in part of glacial origin¹.

Pebble counts and heavy mineral analyses of the channel sediments, the overlying pebbly clay, and several samples of Plateau Drift from the Oxford region, were compared in an attempt to establish their mutual relationships. Quartz, quartzite and sandstone, derived ultimately from the Bunter Pebble Beds, presumably of the Midlands, are major constituents of all three. Flint, derived from the Chalk, possibly when the scarp extended further north than it does today, is common throughout, but limestone of both Kimmeridge and Oolitic lithologies is abundant only in the channel sediments. In contrast, the channel sands and the overlying pebbly clay resemble each other and the Kimmeridge Clay

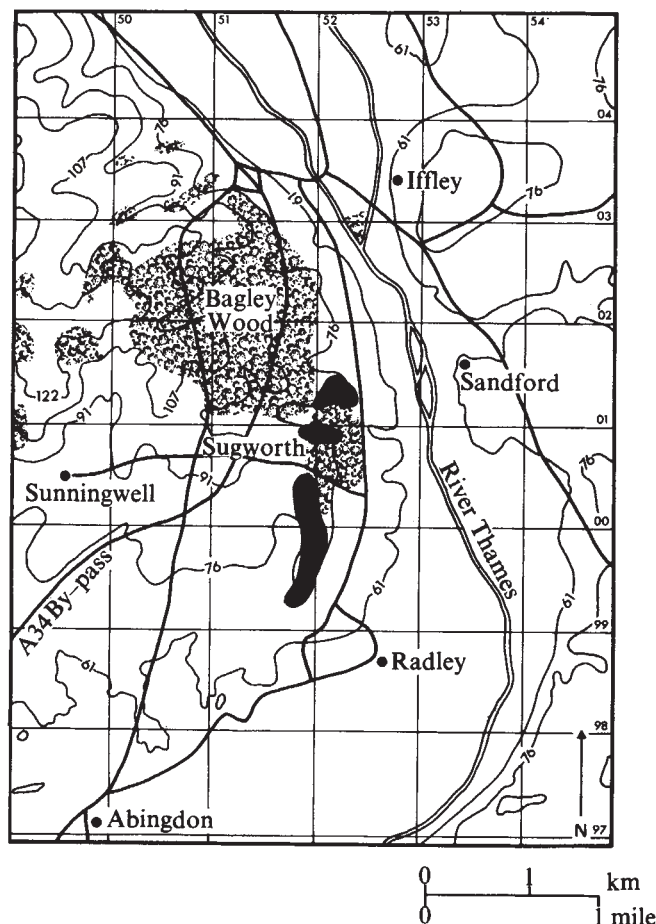


Fig. 1 Map showing location of Sugworth in relation to the Hanborough Terrace (black areas) and the modern River Thames.

in their zircon-dominated assemblage, whereas it has been shown that zircon is rare in the Plateau Drift².

The organic channel sediments are rich in molluscs, beetles and macroscopic plant remains, and vertebrates also occur. Unfortunately, well preserved pollen has not so far been recovered.

The macroscopic plant fossils represents aquatics (for example, *Callitriche* sp. and *Nuphar lutea*), plants characteristic of wet soil (for example, *Lycopus europaeus*, *Eupatorium cannabinum* and *Filipendula ulmaria*), Cyperaceae (sedges), open ground herbs (*Atriplex* sp., *Chenopodium* sp., *Rumex* sp., *Plantago major* and *Leontodon autumnalis*), forest trees (*Acer campestre*, *Alnus glutinosa*, *Carpinus betulus*, *Abies* sp. and *Picea* sp.) and shade tolerant herbs (for example, *Moehringia trinerva*). The logs collected from the deposit are mostly *Ulmus*, together with *Quercus* and a Rosaceae taxon, probably *Crataegus* (hawthorn).

The molluscs from the basal 3 m include many moving water/river species (for example, *Unio crassus*, *Ancylus fluviatilis*, *Valvata piscinalis*, *Bithynia* sp., *Pisidium amnicum* and *P. henslowanum*) reflecting conditions of deposition in a large river. Shells washed in from other habitats include species of reed swamp and fen (for example, *Succinea* spp., *Carychium minimum*, *Cochlicopa lubrica*, *Vertigo angustior* and *Hygromia hispida*) together with a 'slum' group indicative of the presence of small bodies of water, liable to dry up (*Sphaerium lacustre*, *Planorbis leucostoma* and *Pisidium obtusale*), snails of drier rather open terrestrial habitats (for example, *Vallonia costata*) and those favouring a denser forest cover (for example, *Azeca* cf. *menkeana* and *Discus rotundatus*).

Vertebrates include fishes (for example, *Esox lucius*), amphibians (*Rana* sp. and/or *Bufo* sp.) and small mammals (*Sorex* cf. *savini*, *Talpa* sp., *Clethrionomys glareolus*, *Mimomys savini*, *Microtus* sp. and *Apodemus* (sylvaticus group)). Remains of large mammals are few but include a tooth of the rhinoceros *Dicerorhinus etruscus*. The predominance of remains of woodland rodents *Clethrionomys* and *Apodemus* over those of the grassland vole *Microtus* contrasts with assemblages from West Runton³, where the reverse is true, and suggests that forest grew close to the river.

The extensive beetle fauna includes a number of species such as *Hypulus quercinus*, *Eremotes lignarius* and *Dryophthorus corticalis*, which live in deciduous woodland. The general picture, therefore, is of a large river flowing through fairly dense, mixed oak forest, probably with locally developed, herb dominated communities and marshy areas on the floodplain. The channel sediments seem to have accumulated on the inside of a meander.

The faunal and floral evidence strongly indicates a temperate climate and there is no doubt that the deposit is of interglacial age. Some of the plants (for example, *Najas minor*, *Abies* and *Trapa natans*) occur today in central or

Table 1 Known stratigraphical ranges (England) of certain taxa recorded from Sugworth

	Pre-Cromerian	Cromerian*	Hoxnian	Ipswichian
Plants				
<i>Abies</i> sp. ⁸		+	+	
<i>Azolla filiculoides</i> ⁸⁻¹⁰	+	+	+	
<i>Trapa natans</i> ⁸		+		+
Molluscs				
<i>Nematurella runtoniana</i> ¹¹		+		
<i>Valvata goldfussiana</i> †		+		
Mammals				
<i>Mimomys savini</i> ¹²	+	+		
<i>Dicerorhinus etruscus</i> ¹²	+	+		

* Cromerian records are from the type site at West Runton, Norfolk⁷.

† B. W. Sparks, personal communication.

southern Europe. The beetle fauna includes a substantial proportion of species no longer living in Britain but all found further south in Europe today. Examples of this group are *Oxytelus opacus*, *Pelochares versicolor* and *Valgus hemipterus*. There is thus no doubt that the interglacial experienced warmer summer temperatures than the south Midlands of the UK today.

The insect fauna cannot be compared with a definitive Cromerian assemblage as nothing of that age has yet been fully investigated. It is significant, however, that it bears no resemblance to Hoxnian faunas of the Midlands which have been closely studied (ref. 4, and Kenward, unpublished), but there is a marked resemblance to the Ipswichian fauna from Trafalgar Square, London. Nonetheless, a correlation of the Sugworth deposits with the Ipswichian is surely precluded on stratigraphical grounds and on consideration of other fauna and flora with a restricted distribution in time. The known stratigraphical ranges of certain of the Sugworth taxa in deposits of the last three British interglacials (Table 1) cumulatively point to a Cromerian age for the Sugworth channel deposits. More specifically, the abundance of *Carpinus* fruits and the remains of *Abies* and *Picea* suggests that the deposit belongs to zone CrIII. A radiocarbon assay gave an 'infinite' date (Birm 381).

The Sugworth deposit lies topographically well above the Hanborough Terrace (see Fig. 1) which has been ascribed both to the Hoxnian Interglacial and, more recently, to an early part of the Wolstonian⁸. It must, therefore, be at least as old as the Hoxnian and is more

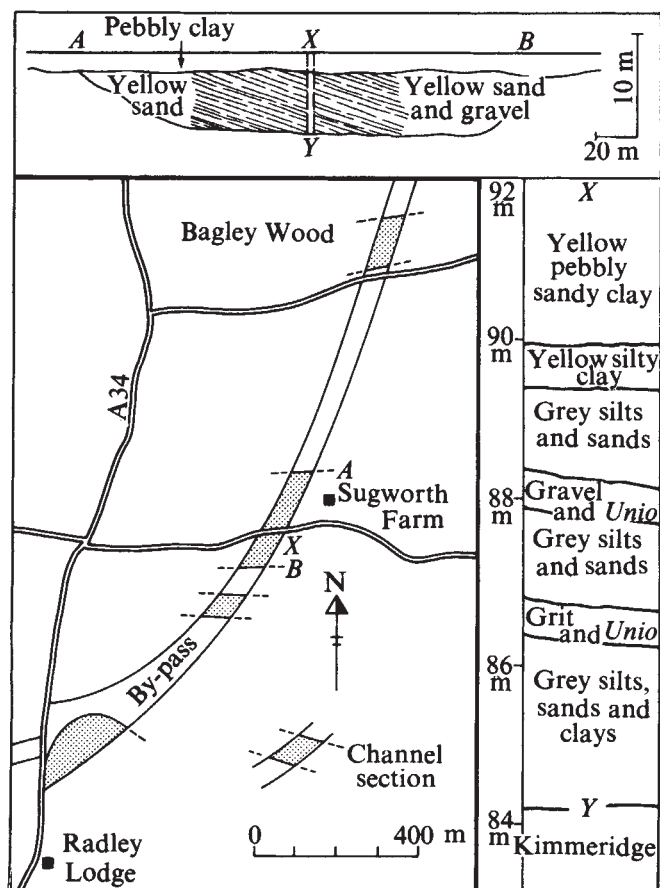


Fig. 2 Location of the channels: idealised section (A-B) of the channel (vertical exaggeration, $\times 5$) with fossiliferous deposits obliquely shaded; detailed stratigraphy, X-Y.

probably even older than that. The evidence that it is Cromerian has wide implications for the Pleistocene stratigraphy of the Oxford region⁶. Of the coarse constituents of the channel deposits, only the Bunter pebbles pose a serious problem since it is possible to suggest a local or near-local source for the other types. If the Bunter pebbles are derived from the Plateau Drift, then that deposit is pre-Cromerian and the pebbly clay which overlies the channel is not Plateau Drift, though it may be a solifluction deposit from it. Since no glacial deposits earlier than the Anglian stage (that is, post-Cromerian) have so far been reliably identified in Britain⁷, the whole question of the mode of deposition of the Plateau Drift, glacial or otherwise, would need clarification were a pre-Cromerian age accepted. If, on the other hand, the pebbly clay above the channel is considered to be Plateau Drift, then that deposit remains in the Anglian stage. In that case, however, a new problem arises: by what mechanism did the Bunter pebbles arrive at Sugworth if earlier ice-transport is ruled out? Possibly, since the channels seem to be part of a river system flowing roughly from the north (assuming that they are indeed relics of a very old river system greatly at variance with the modern pattern) the Bunter pebbles could have been transported from the NNW by fluvial processes. In any case, a minimum distance of transport of 100 km is demanded.

In a wider context Sugworth is important as the only known British open site of Cromerian age outside East Anglia.

We thank the Amey-Fairclough Consortium, the Department of Forestry, Oxford and Dr R. W. Hey for assistance.

A.J.S. acknowledges the support of a Natural Environment Research Council fellowship.

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Adverse effects of maternal alcohol consumption on pregnancy and foetal growth in rats

JONES *et al.* have recorded several combined aspects of dysmorphogenesis, severe physical growth retardation and mental deficiency in the human and named it "foetal alcoholic syndrome", suggesting the defects to be associated with offspring of chronically alcoholic mothers¹. Sandor and Amels² found that ethanol seemed to produce distinct teratological effects in Wistar rats and chick embryos when it was administered intravenously to the pregnant mother or the hen at critical periods in gestation.

In spite of the increasing influence of alcoholism in Western society and the trend toward increased concern for human prenatal health care, no systemic model—human or animal—has been designed to ascertain the adverse effects of maternal alcoholism on the outcome of pregnancy and the growth of the developing foetus. Adequate data in this field are lacking³. This study proposes a simple experimental model to elucidate some information in this area.

Thirty-seven inbred Sprague-Dawley female rats (each about 200 g; Bio-Breeding Laboratories, Ottawa, Canada) were randomly divided into two treatment and one control group, and housed individually in wire cages. For a 5-week period before mating, 13 females (group A) received ethanol in 30 g per 100 ml water as their only available fluid, and a balanced powdered diet (Table 1) *ad libitum*. After 1 month, blood samples were taken from the tails of 10 group A animals for the determination of blood alcohol levels according to the alcohol dehydrogenase method. The

Table 1 Composition of powdered diet (4.2 calorie g⁻¹) fed to all three groups of studied animals

Component	% (by weight)
Casein	20
Corn oil	10
Cornstarch	64
Alphacel	1
Mineral salts	3
Vitamin preparation	2

pair-fed group (B) received water *ad libitum* and were fed a diet isocaloric with that of the group A animals. The isocaloric diet was determined on the basis of the average daily caloric intake of group A animals, in the consumption of both the powdered diet and ethanol. The control group (C) received food and water *ad libitum*.

Food and fluid intake records were kept for all three groups. At mating, three females with one male of the same age, source and strain were placed in each standard plastic breeding cage overnight. All these groups of animals were allowed water during the nights on which they were mated. The presence of sperm cells in vaginal smears taken the following morning was taken as positive indication of pregnancy. Group A animals continued to receive alcohol and the intake of group B animals was regulated accordingly throughout the gestation period. All offspring were examined, weighed and counted on the day of birth.

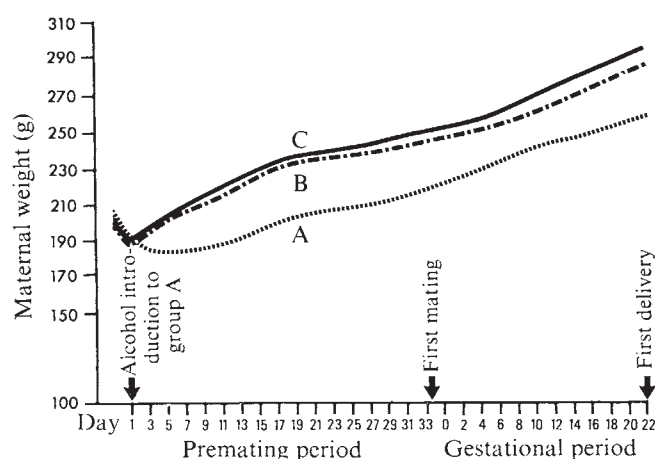
Table 2 Copulation and delivery rates of alcohol-fed, pair-fed and control group females

Group	Number	Positive smears	Successful deliveries
A	12	10 (83%)	5 (50%)
B	13	11 (85%)	10 (91%)
C	11	8 (75%)	7 (88%)

A, Alcohol-treated; B, pair-fed to alcohol-treated; C, control.

Throughout the study period, the daily weight gain of group B females paralleled closely that of control females. Group A animals, after an initial sharp decline in weight with change in environment and start of ethanol administration, gained weight in a pattern similar to that of control and pair-fed females but the group mean weight remained at least 20 g below those of groups B and C (Fig. 1). The mean serum alcohol level of ten group A animals was 61 ± 23 mg %.

No significant difference was found among the three groups with respect to numbers showing positive indications

Fig. 1 Maternal daily weight changes during prenatal and gestational periods in three groups of studied animals. A, Alcohol-fed; B, pair-fed to group A; C, control.

of pregnancy; but whereas 88 and 91% of control and pair-fed females, respectively, which copulated, produced litters, only 50% of group A mothers known to have copulated, delivered litters (Table 2). The average litter size of alcohol-treated mothers (7.2) was significantly lower than the mean litter size of either pair-fed or control mothers in this study. The mean birth weight (4.58 g) of group A litters was also lower than that of litters of group B or C animals (Table 3, Fig. 1). Gestation period did not vary among the three groups; all litters delivered at 21 to 22 d *post coitum*.

In addition to small size, offspring of group A mothers exhibited microcephally, cracked, dry, loose skin, reddened areas on the head and body, and a generally shrivelled appearance.

Table 3 Birth weights of progeny and litter size of alcohol-fed, pair-fed and control females

Group	Mean birth weight (g)	No. of animals per litter at birth
A	4.58 ± 0.23* (N = 34)	7.2 ± 3.4 (n = 4)
B	6.00 ± 0.61 (N = 87)	10.5 ± 3.1 (n = 10)
C	5.88 ± 0.64 (N = 80)	11.7 ± 2.5 (n = 7)

* Mean ± s.d.

N, Total number of offspring; n, total number of litters; A, alcohol-treated; B, pair-fed to alcohol-treated; C, control.

Toews and Lee studied the effect of maternal dietary deprivation of rats on growth and development of progeny⁴, and found that there was no significant effect of nutritional deprivation on litter size, although the mean weight of the progeny was significantly decreased (5.89 g for the offspring of nutritionally restricted animals compared with 6.60 g for offspring of control animals). The difference between the means was 0.71 g. In the present study, the mean weight of progeny in control litters was 5.88 g, whereas that of alcohol-fed litters was 4.58 g. This striking difference of 1.30 g indicates the greater effect of maternal alcohol consumption, rather than dietary restriction, in controlling the size of progeny. Although mothers which were pair-fed to alcohol-ingesting mothers received a somewhat restricted intake of 60 calorie d⁻¹, when compared with *ad libitum* intake of control mothers at 68 calorie d⁻¹, the data show no significant difference between control and pair-fed groups with respect to any of the parameters studied.

Our results indicate that maternal consumption of alcohol before and during pregnancy markedly decreased the size and number of progeny produced. These effects do not seem to be calorie-dependent but rather to be direct effects of the ingestion of alcohol. The simple experimental model used here can readily be applied to other investigations of the adverse effects of maternal alcohol consumption on morphogenesis, foetal growth, postnatal growth and behavioural development of the offspring.

This work was supported by the British Columbia Medical Services Foundation. We thank Miss Bonnie Long for assistance.

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Received June 21; accepted August 19, 1975.

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Alcohol prolongs time course of glare recovery

SUDDEN changes in environmental light levels require the eye to readjust to achieve the same sensitivity to target contrast at the new level, a process which takes many seconds or minutes when the new environment is considerably dimmer than the previous level. During this recovery time, the eye is relatively blind to fine detail. We show here that relatively low doses of alcohol significantly prolong recovery times following bright light exposure; these changes can be seen for several hours following alcohol ingestion.

When the integrity of the retina is disturbed by drugs or disease, the adaptation process is retarded and the recovery of contrast sensitivity is prolonged^{1,2}. Halothane anaesthesia has been shown to markedly prolong the retinal dark adaptation process³. Alcohol, a commonly used drug, is also known to have a direct effect on the human retina; the b wave^{4,5} and the c wave⁶ of the dark-adapted electroretinogram are altered by alcohol at levels commonly encountered in social drinking.

Nine young male subjects (aged from 20 to 28) participated in a replicated 3 × 3 crossover experiment which was run double blind. Two alcohol doses (0.5 and 1.0 ml per kg body weight) and a placebo were used. Alcohol in the form of 95% ethanol was diluted with fruit juice so that the total volume (ml) was three times the subject's body weight (kg). The drink was consumed through a straw from a lidded paper cup which also contained two ice cubes. To minimise olfactory cues to the presence of alcohol, 2 drops each of ethanol and eucalyptus oil were placed on the lid of the cup. Twenty minutes were allowed for the consumption of the drink. Blood alcohol level (BAL) was estimated before drinking and at intervals after drinking using the Intoxilyser (Omicron Systems, Palo Alto) which

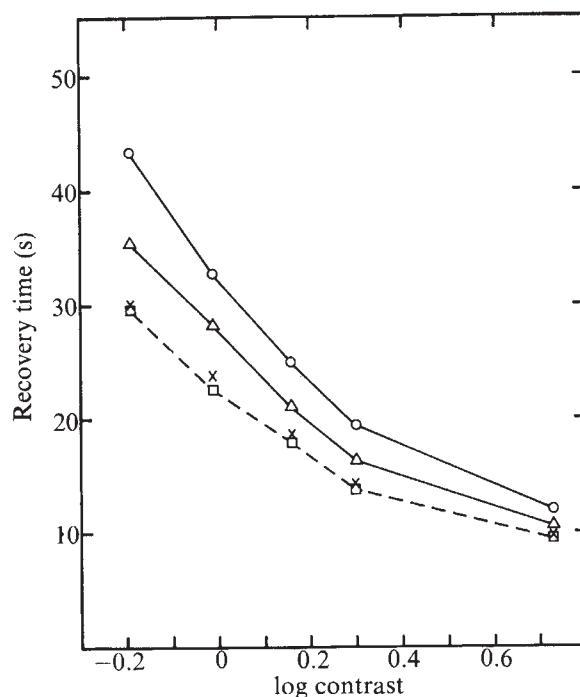


Fig. 2 The time course of adaptation for placebo and alcohol 90 min after drinking. 1.0 ml per kg body weight of 95% ethanol (○); 0.5 ml per kg body weight of 95% ethanol (△); placebo (□). The dose relationship for the alcohol doses is evident in the displacement of the two alcohol treatment curves. The insensitivity of the measure to placebo treatment is also shown here: the mean pre-drink recovery times collapsed across test days (×) fall on the curve generated by the placebo recovery times.

estimates BAL using infrared spectroscopy of a breath sample.

Details of the stimulus presentation are shown in Fig. 1. Subjects practised the task to achieve stable performance before participation in the experimental sessions. On a given experimental day, they were tested before drinking and 30, 90, 180, 270, and 360 min after drinking. At each of these test times, blood alcohol levels and subjective 'high' ratings were ascertained by a second experimenter. Subjects rated their 'high' on a scale of zero to 100, where zero was sober and 100 was as high as a subject had ever been on alcohol.

The time course of adaptation following a 10-s exposure to a uniform bright field is significantly retarded after alcohol

Fig. 3 Group mean recovery time (s) as a function of time after drinking. These functions describe the time course of the alcohol induced light adaptation changes for the five contrast levels and each drug condition. The shaded area indicates the time of alcohol ingestion. Symbols as in Fig. 2.

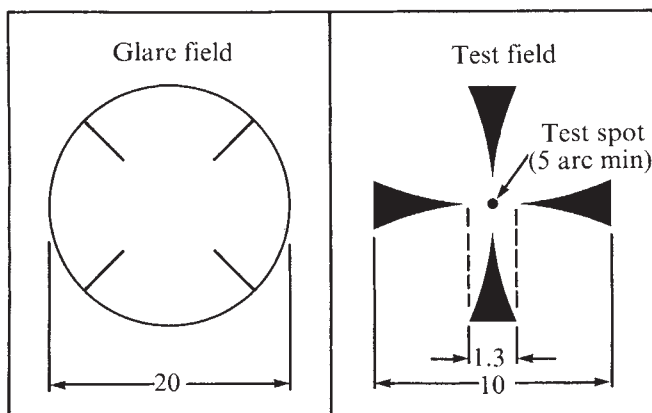
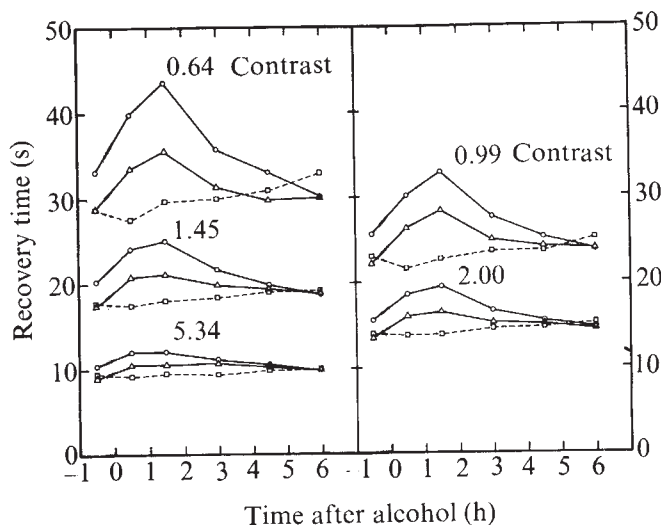


Fig. 1 Glare and test field configuration. Subjects preadapted for at least 5 min to the low photopic luminance levels of the test laboratory. Aided by four thin diagonally oriented reference lines, the subject fixated the centre of a 20° circular adapting field of 5.6×10^4 cd m⁻² with the right eye. Immediately following a 10-s exposure to the high intensity adapting field, the subject fixated the centre of the stimulus configuration where a 5 arc min test spot was intermittently presented (125-ms flashes at 4 Hz) on a 22.6 cd m⁻² background. The contrast of the 5 arc min test spot was controlled by the subject. When he had recovered contrast sensitivity and detected the target, he operated a switch to reduce the target contrast a further fixed step below his threshold. The contrast levels of the test spot, 5.34, 2.00, 1.45, 0.99 and 0.64, were chosen to give approximately equal time periods between each recovery point. Four sets of measurements were taken with 5 min allowed between sets for complete recovery from the adapting luminance level. Photographs of the left eye were taken on five subjects 5 s before and 5 s into the exposure of the high intensity adapting stimulus for the first trial of each test period. Measurements of pupil size were made from the projected negatives.

ingestion. Figure 2 shows the group recovery time to test spots of different contrast for placebo, 0.5, and 1.0 ml of 95% ethanol per kg body weight doses 90 min after drinking. The group recovery times for the low alcohol (0.5 ml kg⁻¹) and high alcohol (1.0 ml kg⁻¹) doses are all increased significantly ($P < 0.02$ Walsh test) when compared with the corresponding pre-drink values. For the 1.0 ml kg⁻¹ alcohol dose, group mean recovery times are delayed by between 30 and 50% compared with the corresponding placebo values.

The alcohol induced increase in recovery times is apparent in the first measurement period (30 min), peaks at the 1–2 h point, and thereafter declines to reach pre-drink values approximately 6 h after drinking (Fig. 3). There is a significant systematic increase ($P < 0.01$, Friedman two-way analysis of variance) in glare recovery time at all five contrast levels for the high dose of alcohol (1.0 ml kg⁻¹); for the low dose of alcohol (0.5 ml kg⁻¹) the shift is statistically significant ($P < 0.05$) for two contrast conditions (2.00, 0.99). Recovery times at all contrasts remain elevated 3 h after drinking.

The alcohol induced increases in recovery times are dose related. The dose relationship is clearly evident 90 min after drinking (Fig. 2). Furthermore, it is evident from Fig. 3 that a dose relationship exists for at least 3 h after drinking when the mean BALs are quite low (0.01 and 0.05 g% for the 0.5 and 1.0 ml kg⁻¹ doses, respectively).

Relatively low doses of alcohol produce significant dose-related delays in the recovery of contrast sensitivity following exposure to intense light levels. The relationship between BALs and the increase in recovery times is illustrated in Fig. 4. Both the mechanisms of the alcohol induced change in the adaptation process and the consequences for a wide range of practical tasks deserve attention.

How does alcohol ingestion produce the increased recovery times? A number of preretinal factors could be involved, including pupil size changes, pupil response to the adapting stimulus, accommodative error, and accuracy of eye fixation.

Fig. 4 Mean recovery time to targets of five contrasts as a function of blood alcohol level. The range of blood alcohol levels was divided into five equal intervals and the corresponding glare recovery times were averaged within these intervals. The number of measures contributing to each point is indicated along the abscissa. Contrast levels: 5.34 (□), 2.00 (△), 1.45 (○), 0.99 (◆), and 0.64 (▽).

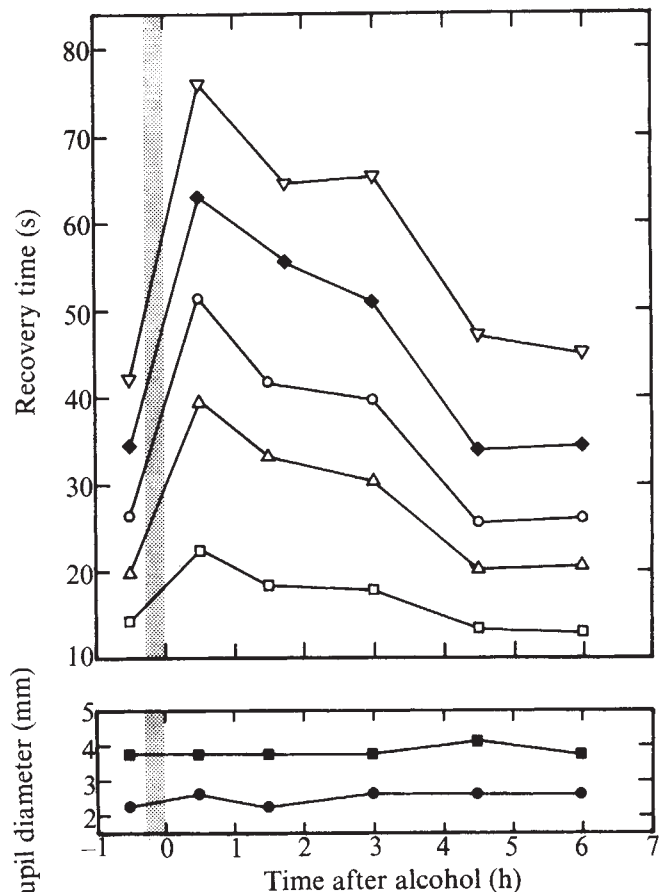
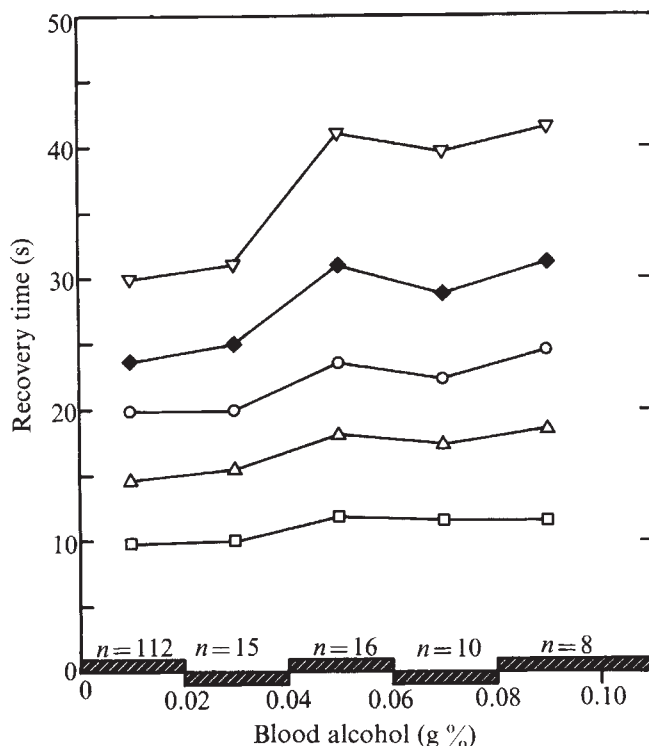


Fig. 5 Recovery time (s) to five different contrasts as a function of time after alcohol (1.0 ml kg⁻¹); subject J.W. Contrast levels: 5.34 (□), 2.00 (△), 1.45 (○), 0.99 (◆), and 0.64 (▽). Shown below is pupil diameter (mm) for this subject before exposure to the glare field (■) and during exposure to the glare field (●) as a function of time after alcohol.

Alcohol induced changes in pupil size could be expected to influence the recovery time to fixed contrast targets. Pupil size showed no consistent variations, however, either with time after drinking or across drug treatments. Figure 5 shows the results for a subject whose glare recovery times were markedly altered by alcohol at all contrast levels. The pupil sizes before and during glare exposure, 90 min after drinking, are no different from their pre-drink counterparts; at this same time there is an average of 54% increase in glare recovery time for the subject. Inaccuracies of accommodation to the test target or inexact foveal fixation could produce longer recovery times. It is unlikely, however, that either of these factors was significant in these experiments. Perhaps the most important evidence for this statement is that visual acuity was unaltered by identical alcohol doses in another experiment on 10 young adult males (Adams *et al.*, unpublished).

Alcohol has a direct effect on the human retina⁴⁻⁶. Further, Raskin *et al.* have shown that alcohol slows dark adaptation and the resynthesis of photopigment in albino rats; their evidence suggests that this is due to the inhibition of a catalyst thought to be required for the oxidative resynthesis of retinaldehyde from retinol⁷. Although some of the evidence is indirect, we feel that preretinal factors and central mechanisms, which have little or no role in light adaptation⁸, are not significantly involved in the alcohol induced slowing of adaptation in our experiments. The results of Raskin *et al.* raise the interesting possibility that alcohol may be inhibiting the resynthesis of photopigment, an hypothesis that needs to be tested by measuring cone pigment kinetics.

The increased recovery time produced by alcohol intoxication must be viewed as critical from a practical point of view,

regardless of the mechanism. The period of recovery is a period of relative blindness for the individual and, as such, is potentially hazardous. Soon after sunrise and just before sunset the sky may act as an extended glare source for the automobile driver. The luminance levels in these conditions may be as high as those experienced by the subjects in our experiment⁹. In certain circumstances a driver will intermittently view the bright sky or be subjected briefly to high luminance glare from light scattered by the windshield. Following glare, important features of the driving environment must be seen against average background levels similar to those which we used. The possible consequences of an additional 30–50% delay in seeing critical detail in such circumstances are obvious.

We have demonstrated alcohol induced delays in glare recovery which we believe to be occurring at the retinal level. These delays have been demonstrated at surprisingly low BALs (approximately one cocktail on an empty stomach) and are dose related. The luminance parameters of the test are similar to luminance levels sometimes encountered in practice.

We thank Dr Alex Cogan, Dr Gunilla Haegerstrom-Portnoy, George Mertz, and Michael Muegge for assistance with this project, which was supported by a grant from the US Army Medical Research and Development Command, to the Visual Sciences Division, Optical Sciences Group.

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Received June 9; accepted August 12, 1975.

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Macrophage proliferation induced *in vitro* by a lymphocyte factor

MACROPHAGE proliferation occurs *in vivo* as a component of the inflammatory response¹ and particularly intense proliferation is characteristic of the delayed hypersensitivity reaction to infection with facultative intracellular pathogens^{2–4}. The thymus-derived (T) lymphocyte is considered responsible for the expansion of the reticuloendothelial system in this type of infection⁵. Although the mechanisms by which lymphocytes induce macrophage proliferation and activation remain unclear, a number of lymphocyte-produced soluble mediators which influence the functions of macrophages have been described, including a biochemically distinct migration inhibitory factor (MIF) and chemotactic factor (MCF)⁶. The speculation that a lymphocyte-produced mitogenic factor may be responsible for the induction and maintenance of proliferation of macrophages prompted the present experiments.

There is evidence that macrophages proliferate *in vitro* in conditioned media or in the presence of a fibroblast-derived growth factor⁷ and two recent reports^{8,9} indicate that inflammatory exudates contain a factor which induces *in vitro* macrophage replication. Here we confirm our preliminary work¹⁰ in demonstrating that guinea pig lymph node lymphocytes in conditions shown to produce intense delayed hypersensitivity produce a soluble factor which induces guinea pig macrophages to proliferate *in vitro*.

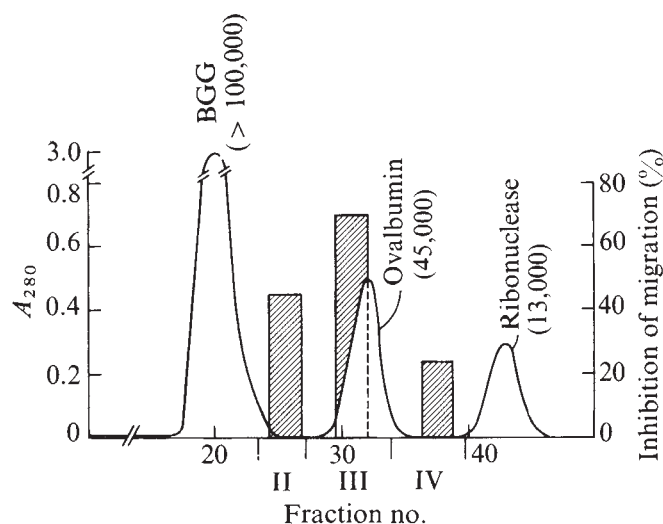


Fig. 1 Sephadex column chromatography of lymphocyte supernatants. P and R supernatants were dialysed, lyophilised and subjected to Sephadex G-100 (superfine) column chromatography as described¹³. Fractions were pooled and assayed for MIF activity on peritoneal macrophages at concentrations equivalent to the undiluted supernatants. MIF activity is expressed as percentage migration inhibition ($[P/R] \times 100$) and depicted as columns. Markers for molecular weight estimation are depicted as curves. Molecular weights are given in parentheses.

Soluble mediators were prepared as previously described by us for the generation of MIF-rich supernatants¹¹. Briefly, Hartley guinea pigs were immunised with bovine gamma globulin (BGG) in Freund's complete adjuvant. Between days 14 and 17 regional lymph nodes were harvested and the cells collected and incubated for 24 h at a concentration of 15×10^6 per ml Eagle's MEM without serum, with and without BGG (1 mg ml^{-1}). After incubation, supernatants were collected and antigen was added to control supernatants. In these experiments, supernatants preincubated with antigen (P) were compared with controls reconstituted with antigen (R). Alveolar and oil-induced peritoneal macrophages from non-immunised animals were used in the capillary tube migration inhibition assay as described¹¹. Supernatants having potent MIF activity at a 1:2 dilution on the peritoneal macrophage (migration inhibition of 60–80%, that is, $[P \text{ migration}/R \text{ migration}] \times 100$) were used in these experiments.

Peritoneal exudate cells and alveolar macrophages were incubated in glass tubes, Lab-Tech chambers or Falcon microculture plates for 30 min at 37°C to allow for the formation of monolayers. Non-adherent cells were removed by decanting followed by three washes in Hanks' balanced salt solution. Monolayers ($> 98\%$ macrophages) were incubated for varying times in medium containing 20% foetal calf serum, 1% L-glutamine, and antibiotics. The incorporation of $0.5 \mu\text{Ci}$ of ^3H -thymidine (20 Ci mmol^{-1}) during

Table 1 DNA synthesis in macrophage monolayers

	Lymphocyte-produced mediator-rich supernatant dilutions	
	1:4	1:2
Alveolar macrophage	3.0 ± 0.3 (9)	4.2 ± 0.4 (12)
Peritoneal macrophage	2.8 ± 0.2 (33)	4.9 ± 0.4 (12)

Because of considerable variation in the background thymidine incorporation between alveolar and peritoneal macrophages and between one animal and another, the thymidine incorporation data for macrophage monolayers incubated for 5 d are expressed as the ratios (P supernatant/R supernatant) $\pm$ s.e.m. of the ratios (number of samples). Mean c.p.m. thymidine incorporation for R supernatants were 7,300 c.p.m. for alveolar macrophages and 3,000 for peritoneal macrophages.

a terminal 24-h pulse was assayed. The tube cultures were processed as described¹². The microculture plates were twice freeze-thawed at -70°C to ensure release of the cells from the monolayer then processed by a multiple automatic sample harvester (MASH, Hiller), followed by liquid scintillation spectrometry.

Monolayers of macrophages incubated in MIF rich supernatants showed significant increases of thymidine incorporation peaking on the average at 5 d of culture. Table 1 shows the results of five experiments in which peritoneal and alveolar macrophages were incubated for 5 d in the presence of 1:2 and 1:4 dilution of P and R supernatants. Negligible decreases in thymidine incorporation were observed with R supernatants compared with media controls. The P supernatants consistently increased thymidine incorporation in both alveolar and peritoneal macrophages. The ratios of stimulation are significant ($P < 0.001$).

Table 2 DNA synthesis and content of macrophage cultures

	48 h		120 h	
	Thymidine incorporation	DNA content	Thymidine incorporation	DNA content
P fraction III	2,839	17.0	20,207	24.8
R fraction III	3,124	17.0	1,381	14.4
Medium control	8,430	18.2	668	16.8

Thymidine incorporation was assayed in microculture in which 1×10^5 peritoneal macrophages were allowed to attach for 30 min at 37°C . Monolayers were washed thrice and incubated for 48 or 120 h. Cultures were terminated with a 24 h ^3H -thymidine pulse ($0.5 \mu\text{Ci}$) and assayed for thymidine incorporation by MASH and liquid scintillation spectrometry. DNA content was assayed in parallel using tube culture in which 5×10^6 macrophages were used for monolayer formation. DNA content of the cultures was measured by the method of Burton¹⁴. Data are expressed as mean c.p.m. or μg of DNA for triplicate determinations in a single experiment.

The crude supernatants contained antigen and possibly small amounts of antigen-antibody complex¹⁵, and since antigen-antibody complexes have been shown to induce macrophage proliferation¹⁶, we fractionated the supernatants on Sephadex G-100 and found that the major portion of the migration inhibitory activity eluted from the column in fractions of estimated molecular weight range of 35–60,000 (fraction III). Similarly, most of the mitogenic activity eluted in this fraction (Fig. 1). Figure 2 shows a time course of the effects of crude supernatants (1:2 dilution) and Sephadex fractions III run at a concentration corresponding to 1:2 and 1:4 dilution of the original supernatants. The increased mitogenic ratio of the supernatants observed in Fig. 2 compared with those given in Table 1 resulted from improving of the culture conditions, particularly a decrease of concentration of CO_2 from 5% to 1%. We have also observed mitogenic activity in fractions eluting before ovalbumin included in fraction II. These results suggest that the mitogenic factor and MIF may represent the same factor; however, we have found that by performing extensive vacuum dialysis of the supernatants before column chromatography (30 h resulting in a 40-fold concentration), we could deplete MIF activity to $< 20\%$ migration inhibition without significantly diminishing the activity of the mitogenic factor. This observation indicates that the mitogenic factor is distinct from MIF.

Table 2 compares the effects of Sephadex P and R fractions III run at a concentration equivalent to an undiluted supernatant and a medium control on thymidine incorporation and DNA content at 2 and 5 d of culture and demonstrates that the mitogenic factor (P fraction III) increases both the thymidine incorporation and DNA content between 48 and 72 h of culture in contrast to the R and medium controls. Microscopic analysis of monolayer cultures during the period of incubation indicated that, compared with R Sephadex fractions, monolayers exposed

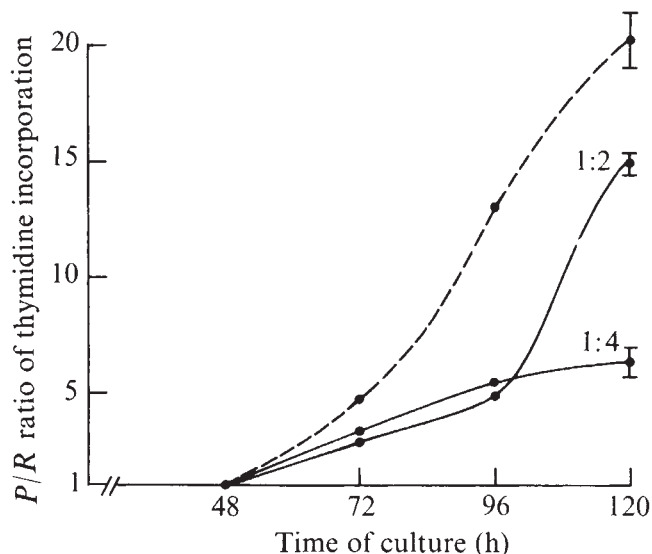


Fig. 2 Time course of mitogenic factor action on macrophage monolayers. P and R supernatants diluted 1:2 were assayed for mitogenic activity on peritoneal macrophage monolayers over 5 d of culture (-----). Comparable supernatants were fractionated on Sephadex G-100 and pooled fractions III (see Fig. 1) were assayed for mitogenic activity at concentrations corresponding to dilutions of the original supernatants of 1:4 and 1:2 (———). Mitogenic activity was determined by thymidine incorporation. The data are expressed as the ratio of P/R thymidine incorporation.

to P fractions containing the mitogenic factor without MIF activity showed no gross differences in the number of adherent cells at plating and during the first 24 h of incubation and marked increases in the number of cells in association with the peak of thymidine incorporation. In an experiment in which the peak thymidine incorporation occurred at 96 h, mean cell counts for monolayers incubated with P fraction III and R fraction III were 47 and 18 cells per high powered field respectively and autoradiographic analyses of cells incorporating ^3H -thymidine were 22.1% and 9.8% labelled cells respectively. The increase in the number of labelled cells having the morphology of macrophages and containing vacuoles filled with oil used to induce the exudate indicate that macrophages were the cells proliferating.

These experiments indicate that lymphocytes in immunological conditions designed to produce intense delayed hypersensitivity produce on re-exposure to antigen *in vitro* a soluble factor which induces the proliferation of macrophages in monolayer cultures. Column chromatographic purification of this factor indicates that this factor elutes with an estimated molecular weight comparable with migration inhibitory factor. Vacuum dialysis before column chromatography indicates that the two factors are different. The factor is also distinct from antigen-antibody complexes, a recognised mitogenic influence on macrophages. On the basis of molecular weight estimation, the factor would seem to be distinct from a lymphocyte produced factor called blastogenic factor (molecular weight 20,000) which is mitogenic for lymphocytes^{15,16} and from a fibroblast-produced factor (molecular weight $> 100,000$) which is mitogenic for peritoneal macrophages⁷. The relationship of this factor to that which induces proliferation in uncharacterised peripheral blood cells remains to be determined. On the basis of comparisons of estimated molecular weight^{17,18}, we estimate that the factor is similar to lymphocyte-produced colony stimulating factor (CSF) which acts on macrophage precursors; CSF has, however, been shown to stimulate colony formation in peritoneal exudate cells only after a delay of 10–14 d and then only in a small fraction of the cells (5%)^{19,20}. To our knowledge, this repre-

sents the first demonstration of lymphocyte-produced macrophage mitogenic factor (MMF) acting on mature macrophages.

The experimental protocol used to elicit MMF offers strong presumptive evidence for its production by T lymphocytes; but whether other cells, for example B lymphocytes, also produce it remains to be determined. Its selectivity of action on target cells remains to be established as well. The biological relevance of MMF seems to be as a participant in the expansion of the expression of the efferent limb of cellular immune response and perhaps in the process of macrophage activation.

This work was supported by grants from the National Cancer Institute and the American Heart Association. John W. Hadden is the recipient of an established investigatorship of the American Heart Association.

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Received May 12; accepted August 19, 1975.

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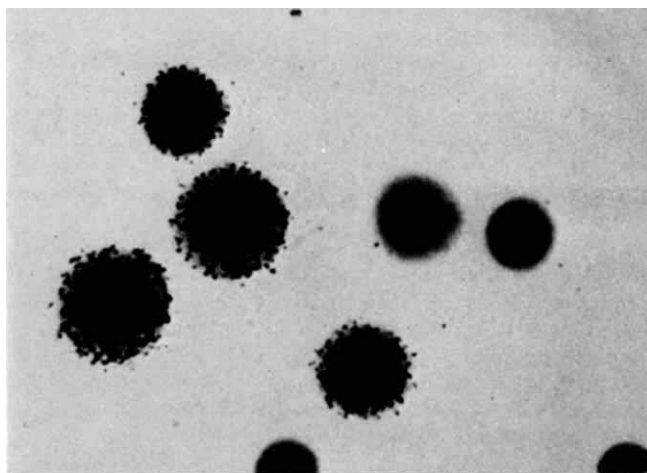


Fig. 1 Autoradiographs of dry smears of human peripheral blood lymphocytes cultured for 48 h in the presence of PHA. Human peripheral blood lymphocytes were obtained from heparinized blood. Culture techniques were based on those described by Moorhead *et al.*⁵ and Hungerford⁶. PHA (reagent grade, Wellcome, UK, reconstituted) was used at a concentration of 0.1 ml per 10 ml culture medium. Cultures were maintained at 37 °C for 48 h. At 41 h, 5 μ Ci methyl-³H-thymidine (5 Ci mmol⁻¹) was added and after 7 h of labelling cells were collected, washed and resuspended in Hanks' balanced solution to a concentration of 1×10^6 ml⁻¹. This suspension, which contained more than 70% lymphoid cells, was split into two. One half was treated according to Moorhead *et al.*⁵ and Hungerford⁶. (Fixing with a mixture of methanol-acetic acid (3:1 v/v) and dry smears of the labelled lymphocytes were prepared. The slides were washed once with 5% Cl₃CCOOH, once with methanol and allowed to dry. The other half of the cultured lymphocytes was mixed with an equal volume of a 0.5% suspension of SRBC and after appropriate incubation (room temperature for 1 h) multilayer rosettes were formed spontaneously. These rosettes remain intact if they were reincubated at 37 °C for 60 min. A multilayer rosette was defined as a lymphocyte surrounded by at least 30 or more SRBC. From these rosettes thin smears were prepared. We used two kinds of control: one with lymphocytes without incubation⁴ and one with lymphocytes incubated for 48 h without PHA before rosetting. The percentage of rosette formation was the same for both, unless they were incubated for 60 min in an ice bath. Furthermore, the rosettes so formed contained only 5-10 SRBC. This observation is in agreement with the findings of Lay and Mendes⁴. The proportion of lymphocytes binding SRBC was found to be 10-15%, whereas Lay and Mendes⁴ have reported 20-40% and Bach *et al.*^{7,8} 0.4-2.6%. Cells were overlaid with a photographic emulsion (Kodak NTB2) and allowed to set for 3 d; after fixation they were counterstained with Giemsa.

DNA present in multilayer rosette formed by lymphocytes stimulated with phytohaemagglutinin

ROGERS *et al.*¹ have reported that human lymphocytes of peripheral blood cultured in the presence of phytohaemagglutinin (PHA) undergo blast formation and synthesise DNA. This newly synthesised DNA which is excreted into the medium is reported to have a high molecular weight. The release of DNA by the cells is selective, as experiments with ¹⁴C-uridine indicated that RNA was not lost into the culture medium. The excretion of DNA by lymphocytes has also been noted by Olsen and Harris². Sarma and Rutman³ also found that PHA-stimulated lymphocytes incorporated labelled thymidine into small fragments that were then converted to high molecular weight material. Subsequently, the labelled thymidine reappeared in light fragments that were then released into the medium in an acid-precipitable form. It has also been reported that the human lymphocytes of peripheral blood are able to bind to sheep red blood cells (SRBC) in a rosette-forming cell⁴ when incubated together in stringent temperature conditions. In view of these findings the release of DNA synthesised by lymphocytes has been studied in connection with rosette formation.

Autoradiographs from the dry (labelled lymphocytes) and

the thin smears (multilayer rosettes) were prepared (for details, see Figs 1 and 2). Figure 1 shows that heavily labelled lymphocytes are present after a 48 h culture period. Up to 80% of the cells become blast-like in appearance and contain ³H-thymidine. Figure 2 shows the formation of multilayer rosettes as a result of incubating SRBC with ³H-labelled lymphocytes. Almost 70% of the lymphocytes formed rosettes, and the striking fact is the almost complete transfer of label from those lymphocytes to the SRBC (Fig. 2). Using human and rabbit erythrocytes instead of SRBC, no rosettes were formed at all, indicating that rosette formation does not depend on the presence of residual PHA on the surface of the blast.

To confirm that intracellular ³H-thymidine was in DNA the labelled cells were collected, taken up in a solution of sodium dodecyl sulphate (1.5%) in 0.01 M Tris buffer, pH 7.4, with 0.01 M EDTA, treated with NaOH at a final concentration of 0.3 M, precipitated with Cl₃CCOOH at a final concentration of 5% and collected on Millipore filters. These were washed twice with 5% Cl₃CCOOH, once with methanol, dried and counted in a scintillation counter. Acid-precipitable ³H-thymidine was determined. Furthermore, the nature of the label observed in SRBC after rosette formation was determined as follows. After culture

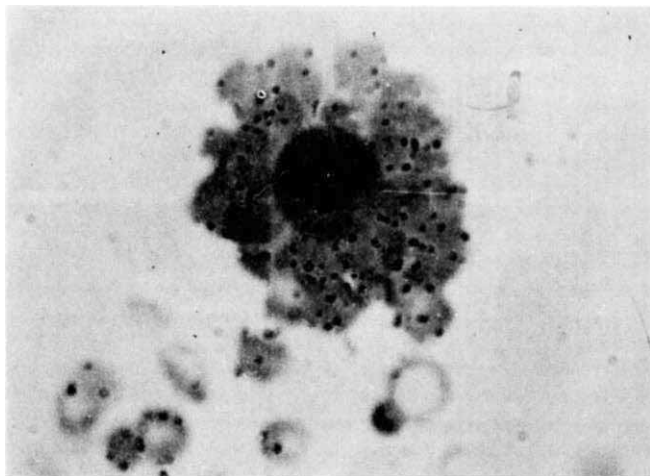


Fig. 2 An equal volume of lymphocytes ($1 \times 10^6 \text{ ml}^{-1}$) and 0.5% suspension of SRBC were mixed. The tubes containing the mixture were incubated at 37°C for 5 min, centrifuged for 5 min at 200g, left at room temperature for 60 min, gently rocked to resuspend the cells in the pellet and thin smears were prepared. Autoradiographs were prepared as described in Fig. 1.

for 48 h duplicate tubes of labelled cultured lymphocytes were collected. On one set acid-precipitable ^3H -thymidine (c.p.m.) was determined as mentioned above; on the other set the cells were incubated with SRBC and rosettes were formed which were centrifuged at 500g for 10 min (supernatant I). The cell pellet was dissolved in distilled water and centrifuged at 5,000g for 10 min (supernatant II). In the cell pellet as well as in the two supernatants, acid-precipitable radioactivity was determined. That found in the labelled lymphocytes (see above) was identical to that in the cell pellet. The two supernatants contain almost zero acid-precipitable ^3H -thymidine radioactivity, indicating that acid-precipitable material is not present in the haemolysate, but bound to the erythrocyte stromata.

Human lymphocytes which had not incorporated ^3H -thymidine into their DNA, do not bind SRBC, although the thin smears were thoroughly scanned. This could mean that these cells have not been stimulated by PHA and are therefore unable to form rosettes. Furthermore, we have noted that PHA-stimulated lymphocytes lose their ability to form rosettes when fixed in a tube with a mixture of methanol-acetic acid (3:1 v/v). In this case lymphocytes were cultured as described previously, fixed in the tube and washed thoroughly in Hank's balanced solution. No rosettes were observed when we used lymphocytes so treated, indicating that cells must be viable to form rosettes.

Lymphocytes undergoing spontaneous transformation excrete DNA in a manner similar to lymphocytes stimulated with PHA and it is likely that this phenomenon is an intrinsic property of the lymphocyte rather than a special function induced by PHA. The observed presence of labelled DNA in the SRBC may reflect the transfer of ^3H -DNA between lymphocytes and SRBC. Although the explanation for the appearance of DNA in SRBC is obscure, a number of possibilities may be considered. Rosette formation is an interaction between a lymphoid cell and a heterologous erythrocyte which, although having some degree of specificity, is probably not immunological in nature. It would be difficult to admit that such a high proportion of peripheral blood lymphocytes (60–80%) have anti-SRBC antibodies on their membranes.

On the other hand the fact that the multilayer rosettes remained intact even at 37°C for 1 h or more is in agreement with the findings of Bach *et al.*^{7,8} for cluster formation between erythrocytes and lymphoid cells from immunised animals. A tentative hypothesis is to consider this phenomenon as a direct cell–cell interaction, which would depend

on the close apposition of large areas of the surfaces of the two cells in a manner impossible to achieve unless both cells were intact and possibly viable⁹. Finally, studies with a long term lymphocyte cell line¹⁰ have shown that about 0.5% of this DNA is associated with the cytoplasmic membrane. Whether these species of DNA are related to the DNA appearing in the SRBC is not known. Further studies on the functional significance of the findings reported here are now in progress.

We thank Professor F. Fessas for reading the manuscript and for discussion.

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Received May 23; accepted July 31, 1975.

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Are contact hypersensitivity cells cytotoxic?

THYMUS-DERIVED lymphocytes (T cells), which participate in many regulatory and effector functions of the immune system, are divided into distinct subpopulations. Thus, T cells causing cell-mediated cytotoxicity can be distinguished from those cooperating with bone marrow-derived lymphocytes (B cells) in the humoral response^{1–3}. Cytotoxic T cells also seem to be distinct from the cells responsible for the mixed lymphocyte and graft versus host reactions^{4,5}. Furthermore, T-cell subpopulations can interact with each other to augment various cell-mediated immune responses^{6–8}. We have investigated whether the T cells causing contact sensitivity belong to a different subset from those causing cell-mediated cytotoxicity. Our studies were based on two observations: first, that in mice contact sensitivity to the trinitrophenyl group (TNP) can be transferred adoptively by spleen or lymph node cells of sensitised animals; and, second, that cytotoxic T cells able to lyse TNP-coupled target cells can be induced *in vitro*^{11–13}. We have found that contact sensitivity cells fail to display cytotoxicity, while cytotoxic cells fail to transfer contact sensitivity. From this we conclude that these two functions are performed by distinct T cell subpopulations.

To induce contact sensitivity to TNP, C57BL/6 mice were sensitised twice by applying 0.1 ml 5% picryl chloride in ethanol to the clipped abdominal skin¹⁴. Five days later their ears were painted with 1% picryl chloride in olive oil and the increase in ear thickness determined after 24 h. Table 1 shows considerable ear swelling in mice sensitised twice, indicating significant contact sensitivity to TNP. After measurement the mice were killed and the draining lymph nodes (axillary and inguinal) were removed, minced, washed and suspended in RPMI-1640 (Microbiological Associates) containing 10% foetal calf serum. Aliquots of 5×10^7 cells were injected into normal C57BL/6 recipient mice, which were challenged immediately with 1% picryl chloride in olive oil applied to the ears. The lymph node cells from these sensitised mice transferred strong contact sensitivity, while the lymph node cells from normal mice did not.

Table 1 Transfer of contact sensitivity to TNP by lymph node cells and their inability to lyse TNP-coupled target cells

Contact sensitivity		Increment of ear thickness at 24 h in 10^{-3} cm $\pm$ s.e.	
Test animals			
Donors: normal mice		7.0 $\pm$ 0.8	
mice sensitised on day -6 and day -11		29.9 $\pm$ 1.7	
Recipients: normal mice		5.0 $\pm$ 0.2	
injected with 5×10^7 normal lymph node cells		6.1 $\pm$ 1.5	
injected with 5×10^7 TNP-immune lymph node cells		15.8 $\pm$ 1.1	
Cytotoxicity		% Cytotoxicity $\pm$ s.e.	
Source of cells	Target cells	4 h	8 h
C57 B16 spleen sensitised to TNP-BALB/c (<i>in vitro</i>)	EL4	0	1.7 $\pm$ 1.6
C57 B16 spleen sensitised to TNP-BALB/c (<i>in vitro</i>)	TNP-EL4	20.4 $\pm$ 1.6	39.5 $\pm$ 3.3
C57 B16 lymph node cells from TNP-C1 sensitised donor	EL4	0	0.3 $\pm$ 0.2
C57 B16 lymph node cells from TNP-C1 sensitised donor	TNP-EL4	0	3.5 $\pm$ 2.7

Donor mice: C57BL/6 mice were sensitised on days -12 and -6 with 100 μ l 5% picryl chloride and challenged on day -1 with 1% picryl chloride on both ears. Ear swelling was determined after 24 h. The mean increments in 10^{-3} cm $\pm$ s.e. are given. Draining lymph nodes were removed and assayed for cell-mediated cytotoxicity at an attacker to target cell ratio of 100:1 on normal and TNP-coupled EL4 target cells¹². As a control, cytotoxic cells able to lyse TNP-EL4 targets were induced *in vitro* by culturing 10^7 C57BL/6 spleen cells with 5×10^4 TNP-coupled BALB/c spleen cells per ml (ref. 12). Spontaneous ^{51}Cr release was 3.2% h^{-1} for EL4 and 3.4% h^{-1} for TNP-EL4 targets. Recipient mice: 5×10^7 lymph node cells from normal and sensitised donors were injected into groups of five normal mice, which were challenged with 1% picryl chloride on their ears, to demonstrate their capacity to transfer contact sensitivity.

Percentage cytotoxicity calculated as:

$$\frac{(\text{experimental } ^{51}\text{Cr release} - \text{spontaneous } ^{51}\text{Cr release})}{(\text{maximal } ^{51}\text{Cr release} - \text{spontaneous } ^{51}\text{Cr release})} \times 100$$

The same lymph node cell preparation was tested for cytotoxicity toward normal or TNP-coupled syngeneic EL4 lymphoma target cells. At an attacker to target cell ratio of 100:1 and within 8 h these lymph node cells were cytotoxic neither to EL4 cells nor to TNP-EL4 cells. By contrast, C57BL/6 spleen cells sensitised *in vitro* to TNP-coupled BALB/c spleen cells¹² killed TNP-EL4 very efficiently, but not EL4, demonstrating that the TNP-EL4 were suitable targets for cytotoxicity. The failure of sensitised lymph node cells to lyse TNP-EL4 cells could have one of three explanations: (1) cells cytotoxic to hapten-coupled target cells are not present in the population responsible for the specific transfer of contact sensitivity to TNP; (2) cytotoxic cells are present in the sensitised population but are inactive (for example, because of suppression); or (3) cytotoxic cells are present but their activity cannot be detected because the assay is not sufficiently sensitive.

To distinguish between these possibilities, C57BL/6 spleen cells were sensitised *in vitro* to TNP-coupled syngeneic spleen cells¹². Table 2 shows that these spleen cells exerted high cell-mediated cytotoxicity on TNP-EL4 targets but no cytotoxicity on EL4 target cells. Various doses of cytotoxic effector cells (5×10^4 – 5×10^7) were injected intravenously into normal C57BL/6 mice, which were immediately challenged with 1% picryl chloride in olive oil on the ears. Table 2 shows that no contact sensitivity to picryl chloride was transferred by any dose of the cytotoxic spleen cells. It seems unlikely that this inability of the cytotoxic spleen cells to transfer contact sensitivity is caused by culturing these cells, because *in vitro* sensitised cytotoxic spleen cells transfer cell-mediated immunity to mice (ref. 15 and our unpublished results). Moreover, *in vivo* sensitised lymph node cells able to transfer contact sensitivity to TNP do not lose this ability after 5 d in culture (our unpublished results). This experiment (Table 2) therefore rules out the third possibility, since cells sufficiently active to be detected by the cytotoxicity assay did not transfer contact sensitivity even at very low doses. The second possibility also seems to be excluded because the population of cells cytotoxic to TNP-EL4 was nonetheless inactive in the contact sensitivity reaction. The first possibility is thus the most likely, implying that cells able to transfer contact sensitivity to TNP can be found in the absence of cells able to cause cytotoxicity to TNP-coupled target cells. Since both cytotoxicity to TNP-EL4 and contact sensitivity to hapten are functions of

T cells^{9–12}, they seem to be properties of two T-cell subpopulations.

Our results seem to conflict with experiments¹⁶ in which lymph node cells of CBA mice sensitised to either oxazolone or picryl chloride were able to lyse the allogeneic DBA/2 mastocytoma P815, provided phytohaemagglutinin was present¹⁶. This suggests that potentially cytotoxic cells are present in the lymph nodes of contact sensitised mice that are absent in normal mice; it does not necessarily mean, however, that the contact sensitivity cells themselves are cytotoxic, since the cytotoxicity could not be demonstrated to be target cell specific¹⁶. Arguing against the

Table 2 Inability of cytotoxic spleen cells to transfer contact sensitivity to TNP

Contact sensitivity		Increment in ear thickness at 24 h in 10^{-3} cm $\pm$ s.e.	
Test animals			
Normal mice		3.6 $\pm$ 0.4	
Mice sensitised on day -6		13.0 $\pm$ 1.3	
Recipients of 5×10^7 non-immune spleen cells		4.8 $\pm$ 1.5	
5×10^7 immune spleen cells		4.9 $\pm$ 1.4	
5×10^6 non-immune spleen cells		3.8 $\pm$ 1.1	
5×10^6 immune spleen cells		4.2 $\pm$ 1.6	
5×10^5 non-immune spleen cells		3.9 $\pm$ 1.7	
5×10^5 immune spleen cells		3.5 $\pm$ 0.9	
5×10^4 non-immune spleen cells		5.0 $\pm$ 2.4	
5×10^4 immune spleen cells		4.3 $\pm$ 1.2	
Cytotoxicity		% Cytotoxicity $\pm$ s.e.	
Source of cells	Target cells	4 h	8 h
C57 B16 spleen sensitised to TNP-C57 B16 spleen	EL4	0	0
C57 B16 spleen sensitised to TNP-C57 B16 spleen	TNP-EL4	16.4 $\pm$ 1.4	48.1 $\pm$ 3.7

C57BL/6 spleen cells (10^7 ml^{-1}) were sensitised *in vitro* to TNP-C57BL/6 spleen cells ($5 \times 10^5 \text{ ml}^{-1}$)¹². After 5 d cultures were collected and cytotoxic activity assayed on EL4 and TNP-EL4 target cells at an attacker to target cell ratio of 100:1. Spontaneous ^{51}Cr release was 2.9% h^{-1} for EL4 and 3.1% h^{-1} for TNP-EL4 targets. Spleen cells of cultures incubated with or without antigen were injected in various doses into groups of five normal recipients which were challenged with 1% picryl chloride. As controls, normal mice and mice pre-sensitised once with picryl chloride on day -6 were included.

identity of contact sensitivity and cytotoxic cells is the finding that resensitisation of mice decreases the cytotoxic activity in the lymph nodes, while it increases their potential to transfer contact sensitivity (ref. 16 and our unpublished results).

It could be argued that the hypersensitivity cells are cytotoxic but do not lyse the TNP-EL4 target cell because they do not recognise the TNP group, but rather some larger moiety, including the determinant to which TNP is coupled. Evidence for this possibility has been presented with respect to the guinea pig^{17,18}. Recent experiments, however, have shown that cytotoxic mouse spleen cells, able to lyse the TNP-EL4, can recognise both the TNP group and TNP-modified self antigens¹¹⁻¹³ so that the failure of such cytotoxic cells to demonstrate contact sensitivity *in vivo* cannot be ascribed to inability to recognise the TNP group.

On the assumption that antigen recognition in the contact sensitivity reaction is similar to that in the cytotoxic reaction, our conclusion is that these reactions are performed by two distinct T-cell subpopulations.

This work was supported by grants from the National Cancer Institute. We thank Margaret DeRose for technical assistance.

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Received April 3; accepted August 4, 1975.

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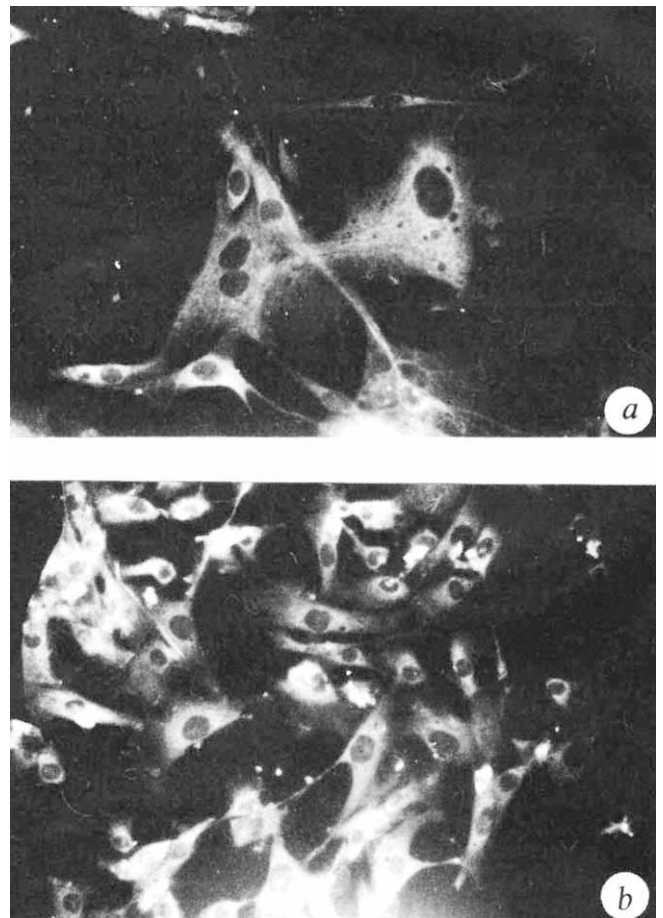


Fig. 1 Positive indirect immunofluorescence test for rat foetal antigens; ultraviolet light. *a*, Methanol-fixed DON Chinese hamster cells (from adult lung; American Type Culture Collection) 8 d after exposure to rat spermatozoa ($\times 248$). *b*, Methanol-fixed colony of DON cells 48 d after exposure to rat spermatozoa ($\times 157$). Cells were examined by the indirect immunofluorescence technique¹² by treating with Chinese hamster tissue-absorbed rabbit antiserum prepared against lyophilised skin-free trunk extracts of 17–21-d rat fetuses. After washing, the cultured cells were then stained with fluorescein-conjugated goat anti-rabbit IgG which had previously been absorbed with both Chinese hamster tissue extract and rat liver extract.

Appearance of foetal antigens in somatic cells after interaction with heterologous sperm

GENETIC traits may be transferred to tissue cultured mammalian cells by interaction with viruses^{1,2}, nucleic acids^{3,4}, and cellular organelles such as metaphase chromosomes⁵⁻⁷, and by other cells^{3,8}. Recent findings⁹ indicate that incubation of mouse spermatozoa with Chinese hamster bone marrow fibroblasts (CLM) results in uptake of sperm, morphological alterations, and, at a frequency of about 0.01%, subsequent mouse foetal antigen expression in the heterologous fibroblast progeny. To learn whether the efficiency of these processes can be enhanced, combinations of several species of sperm and target cells were studied in orienting experiments¹⁰.

The interaction of rat spermatozoa with Chinese hamster lung fibroblasts (DON) in tissue culture led to a higher yield of altered cells than was observed in the CLM-mouse sperm system. For collection of spermatozoa, the vasa deferentia were excised aseptically from adult Wistar strain rats, placed in Brackett's solution¹¹ plus 5% foetal bovine serum (FBS), and the living sperm were gently extruded into the medium without mincing the vas deferens. The sperm were separated from any remaining tissue clumps and washed by brief low speed centrifugation in this medium and adjusted to a concentration of 3×10^7 sperm

ml⁻¹. A suspension of 8×10^5 DON cells in 1.6 ml of Ham's F12 medium which contained 2% heat-inactivated FBS was mixed with 0.4 ml of the above sperm suspension. The ratio of sperm to cells was thus 15:1. After 2 h at 37 °C, 0.4 ml aliquots of the mixed cells were pipetted into 60-mm Petri dishes which contained microscope cover slips, and 4.6 ml of F12 medium plus 20% heat-inactivated FBS was added to each. Incubation was at 37 °C in a moist atmosphere of 5% CO₂ in air, and coverslips were withdrawn for examination after various times³. Control cultures of DON cells were incubated in parallel in the same final medium. Cell morphology was studied microscopically after fixation of monolayers with methanol and staining with Giemsa. By 48 h after admixture, about 50–55% of the DON cells seemed to have been penetrated by at least one spermatozoon. Colcemide-treated cultures were used to study metaphase chromosomes at various times after the first day of admixture.

Control DON cultures maximally showed a 3% incidence of binucleate cells and an occasional cell with three or four nuclei. In contrast, the fibroblasts in the mixed cultures showed a higher proportion of multinucleates beginning about 24 h after exposure to sperm, and at 96 h 14% were multinucleate. Although binucleation was the major morphological change in the 96-h mixed cultures, many of the

sperm-treated cells had four to six nuclei per cell. The proportion of cells in sperm-treated cultures with an approximately tetraploid number of chromosomes was also higher than in control populations. The modal chromosome number of the DON cells in control cultures was 22. Two days after exposure to rat sperm, 8% of the treated cells contained 40 or more chromosomes. Parallel control cultures at this stage had a 2% frequency of tetraploid cells and these possessed 44 chromosomes. The incidence of near tetraploids (cells with 40–50 chromosomes) 3 d after exposure to sperm had increased to 14%. Control values of tetraploid cells never exceeded 3% during the course of this study. Statistically, the increase over control values seen in rat sperm-treated cultures was highly significant, $P < 0.01$.

Progeny Chinese hamster cells at various times after interaction with spermatozoa were examined for the presence of rat antigen by the indirect immunofluorescence technique¹² using antiserum prepared in the rabbit against lyophilised preparations of saline extracts¹³ of days 17–21 Wistar rat foetal tissue, and staining with fluorescein-conjugated goat anti-rabbit IgG. The rabbit antiserum against rat foetal tissue extracts and the goat anti-rabbit IgG were absorbed with lyophilised extracts and whole tissue homogenates of adult Chinese hamster organs. The conjugated goat anti-rabbit IgG was further absorbed with lyophilised extracts of rat liver. At day 8, but not before that, approximately 0.5–1.0% of the cells examined in the ultra-violet showed positive cytoplasmic immunofluorescence (Fig. 1a). Of the positive cells, 18% had more than one nucleus, with binucleates predominating in this group; about 10% of the positive cells had a vacuolated cytoplasm. At days 14 and 36 after interaction with sperm, the proportion of progeny cells which exhibited positive cytoplasmic immunofluorescence increased to about 3% and 4% respectively. At day 39, cells were lightly seeded on coverslips to obtain individual colonies; after 9 d, all the cells in 1–2% of these colonies showed positive immunofluorescence (Fig. 1b).

Control DON cells and the washed rat spermatozoal preparations used were consistently negative with both immune and pre-immunisation rabbit serum; sperm-treated DON cells were negative throughout the 48-d period when tested with preimmune serum. Cultured Fisher rat embryo cells were positive with immune serum, but cultured adult Gunn rat liver cells¹⁴ and Morris hepatoma cells were negative. Rabbit antiserum against whole adult rat serum, after absorption with Chinese hamster tissue as above, gave positive immunofluorescence tests with the rat liver cells, but the tests were negative when applied to DON cells before and after mixture with rat sperm. Contaminating rat somatic cells in the sperm preparation would have been revealed in this test.

Experiments are under way to isolate and characterise the component(s) responsible for the immunofluorescence in individual positive clones, and to learn whether tumorigenicity can be demonstrated. In previous studies^{3,4}, synthesis of mouse antigens continued in CLM cells for at least a year of growth after interaction with mouse tumour cells or with DNA isolated from the latter; oncogenic potential was also acquired.

Although rigorous evidence that transcription and translation of rat sperm genetic material occurred in the DON population is not yet provided in these ongoing studies, it is unlikely that the results reported could have been due to rat somatic cells which had contaminated the sperm. Karyotype analyses never revealed the presence of rat chromosomes in hundreds of metaphase plates examined after coculture with the rat sperm. Immunofluorescence tests at times earlier than 8 d were consistently negative for rat foetal antigen. Furthermore, somatic cells of rat origin did not propagate when the sperm preparations themselves were subjected to the tissue culture conditions

used. We conclude that the intense cytoplasmic fluorescence observed after the interaction of rat sperm and DON cells was a result not of the presence of rat somatic cells or sperm proteinaceous material, but of the synthesis of rat foetal protein by the hamster cells.

The immunofluorescence data indicate that rat protein, most probably of foetal nature, persisted for at least 40–50 generations in Chinese hamster cells after mixture with rat sperm. Some proteins regarded as characteristic of the foetal stage of ontogeny are known to reappear in the adult just before the onset of and during malignant growth^{15,16}.

The data presented provide additional evidence that mammalian spermatozoa are capable of penetrating somatic cells *in vitro* and of affecting morphology and the normal process of mitosis. Independent confirmation of such penetration has appeared^{17,18}. The results in the present report suggest that sperm can act as vectors for the transfer and subsequent expression of genetic information in heterologous somatic cell systems. Whether the results are a consequence of a fertilisation-type reaction or are due to the presence of a recently discovered RNase-sensitive endogenous DNA-synthesising complex within sperm heads¹⁹, or to a still unknown mechanism is not yet clear. It is hoped that use of the sperm-somatic cell system will provide a further understanding of the significance of foetal proteins in oncogenesis^{20,21}.

We thank M. Steinglass for assistance, and Drs C. Tong and S. Witkin for discussions. This work was supported in part by a Public Health Service grant from the National Cancer Institute and a contract from the National Cancer Institute.

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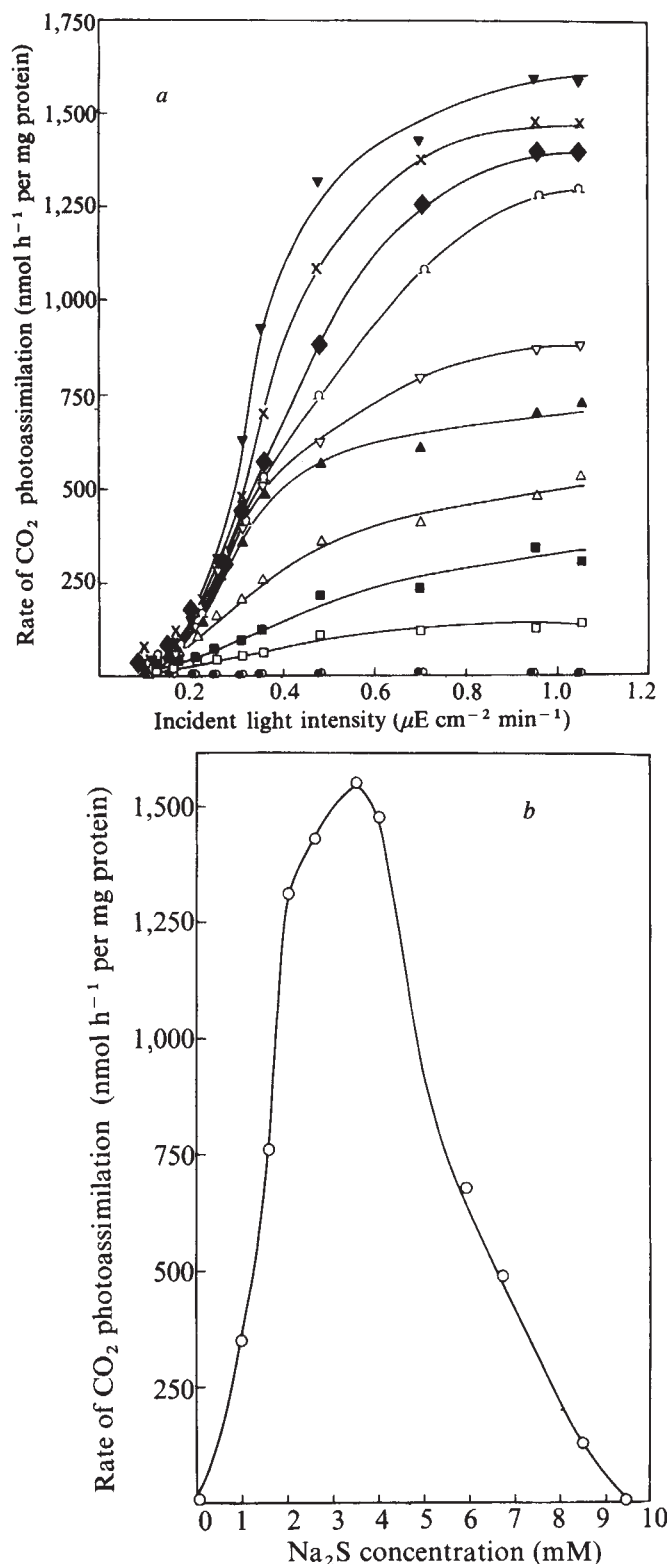
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Sulphide-dependent anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*

PROFOUND differences in structure and function separate the prokaryotic cyanobacteria (blue-green algae) from eukaryotic algae and plants, whereas oxygenic photosynthesis is considered to distinguish the cyanobacteria and eukaryotic plants from the



other phototrophic bacteria. The prevailing concepts on the cyanobacteria emphasise their prokaryotic nature and oxygenic photosynthesis. This combination of properties, among others, led to the suggestion that the cyanobacteria represent a group of possible progenitors of chloroplasts¹. While cyanobacteria typically exhibit oxygenic photosynthesis with two photosystems using electrons from water, it has been demonstrated that photosystem I of certain cyanobacteria can function *in vivo* independently in supporting virus production², heterocyst functions³ and even photoheterotrophic growth⁴. We have demonstrated anoxygenic photosynthesis in a cyanobacterium *Oscillatoria limnetica*, also capable of oxygenic photosynthesis,

Fig. 1 *a*, CO₂ photoassimilation by *Oscillatoria limnetica* in the presence of DCMU at different Na₂S concentrations and intensities of 704-nm wavelength light. Twice-washed, logarithmic phase cells were suspended in the growth medium (15 μg cell protein per ml) at various Na₂S concentrations with DCMU (5 μM) in 5-ml stoppered vials and preincubated for 2 h at 35 °C in light provided by a 60-W tungsten lamp (incident intensity of 2×10^4 erg cm⁻² s⁻¹). NaH¹⁴CO₃ was introduced to a final specific activity of 1.50 μCi μmol⁻¹, and the suspension incubated for 5 min under the actinic light provided by a Prado Universal-Leitz projector and filtered through a Corning HR 2-60 filter and then Schott Mainz sharp cutoff interference filter blocked to infinity peaking at 704 nm (11-nm half bandwidth). The incident intensity of the actinic light, varied by using a powerstat, was measured by a Yellow Springs Instrument Radiometer model 65. The cell suspension was filtered on glass filter paper (Whatman GF/C), washed with 40 ml 0.6 M trichloroacetic acid (4 °C), and cell radioactivity was counted by a gas-flow counter. Filters were washed with 5 ml of absolute ethanol to remove elemental sulphur, and protein was determined⁹. Various Na₂S concentrations in mM, were determined (methylene blue photometric method¹⁰) when the radioactivity was added. Concentrations (mM): ▼, 3.5; ×, 4.0; ◆, 2.8; Ω, 2.0; ▽, 1.6; ▲, 5.9; △, 6.7; ■, 1.0; □, 8.5; ●, 0.0; ○, 9.5. *b*, Plot of photoassimilation rate (from *a*) at the saturation light intensity (1.0 μerg cm⁻² min⁻¹) as function of Na₂S concentration.

which was isolated from the anaerobic H₂S rich hypolimnion layer of the monomictic, hypersaline Solar Lake located on the desert margin of the Gulf of Elat, Israel⁵. During winter stratification, this layer is located below two plates of phototrophic sulphur bacteria (*Chromatium violescens* and *Prosthecochloris* sp.), and exhibits a dense cyanobacterial bloom with very high rates of primary production. We report here evidence for the photosystem I-driven CO₂ photoassimilation in *O. limnetica* with H₂S serving as sole electron donor through oxidation to elemental sulphur.

O. limnetica was grown in pure culture in a mineral medium prepared in Solar Lake water (96.7‰ chlorinity; 174.1‰ salinity), containing the major elements (g l⁻¹): KH₂PO₄, 0.33; NH₄Cl, 0.33; MgCl₂·6H₂O, 0.33; KCl, 0.33; NaHCO₃, 1.50; Na₂S·9H₂O, 0.075; vitamin B₁₂, 10⁻⁶; as well as SL 4 trace elements⁶; final pH was adjusted to 6.8 with HCl. Cultures were grown in completely filled glass-stoppered bottles, illuminated continuously by white fluorescent lamps (4,300 K, 20 W, incident light intensity 8×10^3 erg cm⁻² s⁻¹) at 35 °C.

The ability of the cyanobacterium to photoassimilate CO₂ in reactions driven by photosystem I alone and using Na₂S was demonstrated in the presence of DCMU under actinic light of 704-nm wavelength (Fig. 1*a*); no photoassimilation was observed in the absence of sulphide or light. Although the detailed mechanism of the sulphide oxidation is not known, the photosystem I-driven reaction and use of Na₂S are similar to that observed in many phototrophic sulphur bacteria. This photoassimilation is saturated by 704-nm wavelength light at an incident intensity of about 1.0 μerg cm⁻² min⁻¹. The plot of the photoassimilation rate at light saturation as a function of sulphide concentration shows an optimum around 3–4 mM Na₂S (Fig. 1*b*).

The fate of the sulphide, oxidised during the Na₂S-photo-system I-driven CO₂ photoassimilation in *O. limnetica* was determined from the distribution of the radioactive label in different sulphur fractions of the culture incubated with Na₂³⁵S in the presence of DCMU (Fig. 2). The decrease in ³⁵S²⁻ is accompanied by an almost equivalent increase in ³⁵S⁰. The small increase in ³⁵S₂O₃²⁻ and ³⁵SO₄²⁻ together are accounted for by chemical sulphide oxidation (as observed in the cell-free control). It seems that *O. limnetica* photo-oxidises S²⁻ quantitatively to S⁰. The rate of Na₂S oxidation in this experiment in the 10–25-h interval was 3.7 μmol Na₂S per mg protein per h; the rate of CO₂ uptake measured in identical conditions was 1.8 μmol CO₂ per mg protein per h. The stoichiometric relationship between CO₂ photoassimilation and H₂S oxidation derived, suggests the following equation:



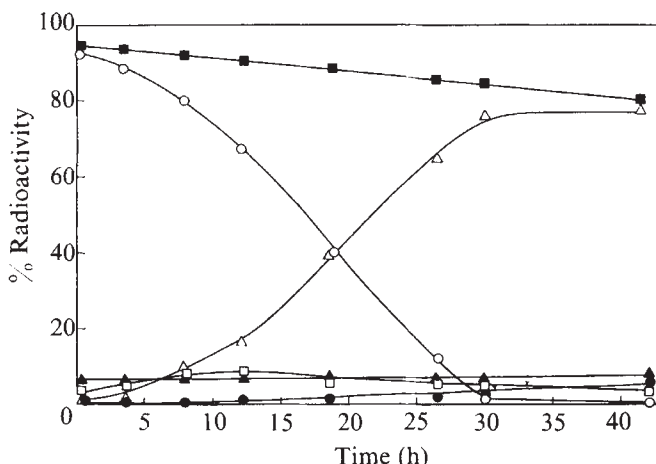


Fig. 2 Sulphide photo-oxidation by *Oscillatoria limnetica*. Cells, as described in Fig. 1, were suspended (25 µg cell protein per ml) in growth medium and flushed thoroughly with oxygen-free N₂. Cells were preincubated in the dark at 35 °C for 2 h, after which Na₂³⁵S was added anaerobically to a final specific activity of 0.1 µCi µmol⁻¹ and then incubated at 35 °C in continuous light provided by a 40-W tungsten lamp at a distance of 40 cm. Dark control and blank (without cyanobacteria) for chemical sulphide oxidation were run in parallel; in both cases sulphide oxidation did not exceed 12% throughout the experiment. Samples (5 ml) were filtered in a Swinnex filter holder through a 0.45-µm (average pore size) membrane filter directly into 2 ml of 0.11 M CdCl₂; filters were washed once with 10 ml of oxygen-free water, which was added to the filtrate (soluble S²⁻ fraction). The filter retaining both the particulate S⁰ and organic S fractions was homogenised in distilled water (5 ml) and transferred to a scintillation vial. These latter fractions were separated in another 5 ml sample of the experimental suspension. S⁰ and pigments were extracted by filtering the suspension and treating the glass filter with 5 ml of absolute ethanol, and were determined photometrically in the filtrate¹⁰. The filter retaining the organic S fraction was homogenised and transferred to a scintillation vial. The soluble S²⁻ fraction together with 0.1 nmol CdS carrier was transferred to an apparatus for anaerobic distillation¹¹. After flushing the apparatus with N₂, 10 ml of oxygen-free 6 N HCl was added. Sulphide was distilled into 10 ml of 0.1 M Zn acetate at room temperature with N₂ as carrier gas; 5 ml of the suspension of the ZnS produced was transferred to a scintillation vial for counting the ³⁵S²⁻ fraction. To precipitate sulphate, 5 ml of 2 M BaCl₂ was added to the undistilled remaining solution, diluted to 50 ml with water at room temperature. A 5-ml sample was transferred to a scintillation vial to determine both ³⁵SO₄²⁻ (as Ba³⁵SO₄) and dissolved ³⁵S₂O₃²⁻; the radioactivity in the remaining filtrate represented only the S₂O₃²⁻ fraction, although this was not demonstrated chemically. Insta-gel scintillation liquid (5 ml) was added to all the vials for counting in a Tri-carb scintillation counter. Radioactivity determinations of the various sulphur fractions are expressed as percentage of total sulphur at each sampling time. Experimental variation was no more than ±5%. ■, Blank H₂S; △, S⁰; ○, H₂S; ▲, S₂O₃²⁻; □, SO₄²⁻; ●, Organic S.

Elemental sulphur excreted from the cells was observed as typical refractile granules adhering to the cyanobacterial filaments (Fig. 3) or in the medium. The photosynthetic sulphur oxidation of Na₂S is similar in this respect to that of the Chlorobiaceae and some Chromatiaceae. In addition to the anoxygenic photosynthesis described here, this *O. limnetica* strain is capable of oxygenic photosynthesis and can readily shift to growth with either type of photosynthesis. The special physiological capacities of this strain represent an ecological advantage in an ecosystem such as the Solar Lake with an annual limnological cycle in which oxygenated conditions during holomixis alternate with high sulphide concentrations during stratification. *O. limnetica* is distributed throughout the lake at holomixis when the phototrophic bacteria disappear and thrives during stratification in the bottom layers, where the sulphide content is extremely high (40 mg H₂S per l) and prohibitive for the growth of the two other phototrophic bacteria.

The potency for using H₂ and other reducing compounds as electron donors for photoreduction of CO₂ has been demonstrated in special experimental conditions in certain oxygenic phototrophic organisms⁷, but growth was not supported. Attempts to grow other cyanobacteria in anaerobic conditions utilising H₂S as sole electron donor demonstrated that photosystem II is required⁸. *O. limnetica* represents the first case of an oxygenic phototroph that can grow anaerobically on H₂S. The significance in the evolution of oxygenic photosynthesis of a prokaryotic organism with both types of photosynthesis is highly suggestive. This strain also offers new possibilities for isolating photosystem II mutants.

We thank M. Avron of the Weizmann Institute for stimulating discussions and suggestions. A. Oren participated in the experimental work, M. Kessel provided the photographs and Binah Golek helped in the writing. This work was supported by a grant from the Deutsche Forschungsgemeinschaft.

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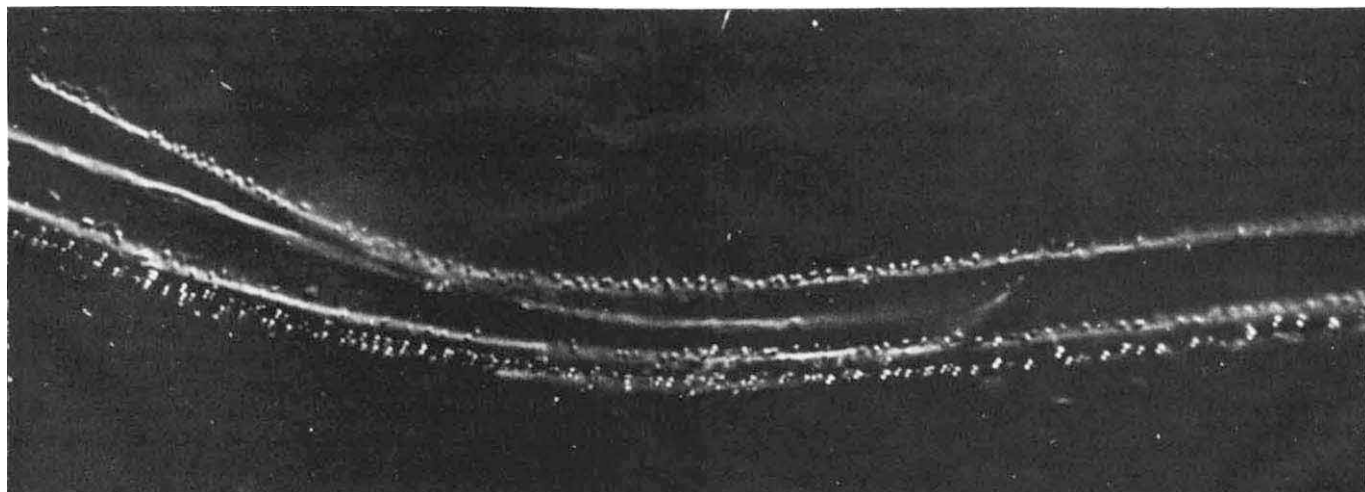


Fig. 3 *Oscillatoria limnetica* filament grown in anoxygenic conditions. Refractile sulphur granules are seen adhering to filaments. Nomarski interference contrast ($\times 2,558$).

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Light inhibits the labelling of gangliosides in chicken retina

We have reported that the labelling of brain gangliosides is more extensive in chickens given labelled glucosamine and subsequently exposed to light than in other chickens kept in the dark¹. The opposite result was obtained with rat brain². These effects parallel the influence of light on the general activity of the animals, and we could not decide whether the cause was a direct or indirect stimulus on the labelling process. Similar studies with the retina may help to solve this problem, for the retina is stimulated directly by light, and it is possible, by occluding one eye, to measure labelling in different conditions of illumination in one animal. We have now done such experiments and found that the labelling of retinal gangliosides varies in response to light: these changes are not attributable to systemic effects.

Labelling of retinal gangliosides in chickens maintained in the dark was greater than in those exposed to light (Table 1, experiments 1 and 2). Results were similar when one eye was occluded (experiment 4). In the latter case the difference was smaller and so the possibility remained that in experiments 1 and 2, a general effect was added to that of the incident light. But a substantial direct inhibition by light of retinal ganglioside labelling seems to be proved by our data.

Values for ganglioside labelling given in experiments 1 and 2 were normalised by the radioactivity of the acid-soluble fraction, but these ratios do not reveal the interesting fact that there was no difference between the acid-soluble fractions from the two groups of animals. The radioactivity of the acid-soluble fraction is shown in experiments 1a and 2a of Table 1. These results suggest that light did not affect the permeability to

labelled glucosamine or its derivatives, unless there was some effect in very limited areas of the retina.

The difference in the labelling of the sialyl moiety was at least partially responsible for the change in the gangliosides (Table 1, experiment 3). On the other hand, no individual ganglioside could be singled out as being specifically affected by light and this was investigated by cochromatography. Chickens kept in the light or dark received, respectively, injections of ³H- or ¹⁴C-glucosamine or vice versa. Two hours later they were decapitated and the retinae pooled and minced. The gangliosides obtained from this pool were run on thin-layer chromatography and the ³H/¹⁴C ratio was determined every 0.5 cm of the chromatogram. In seven experiments we could not identify a zone with a ratio of labelling significantly different from the others.

There have been reports of the hyperpolarisation of the photoreceptor membranes in the vertebrate retina exposed to light, compared with that in the dark (for a review see ref. 3). It has been speculated that this hyperpolarisation interrupts the secretion of neurotransmitter from the synaptic endings⁴. For gangliosides, the precise step inhibited by light is unknown, but their participation in a presynaptic phenomenon is suggested by the high affinity of gangliosides for toxins of the presynaptic effect, such as tetanus and botulinum toxins^{5,6}.

In chickens another striking observation is that whereas exposure to light inhibited the labelling of gangliosides in the retina, it stimulated labelling in the telencephalon¹ the possibility that inhibitory events lead to decreased ganglioside labelling in the chicken retina exposed to light and that excitatory events lead to an overall increase in brain ganglioside labelling is worthy of investigation. The effect seen in the rat brain², which is opposite to that observed in chicken brain, requires explanation.

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Received February 24; accepted July 4, 1975.

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Table 1 Labelling of gangliosides, gangliosidic sialic acid and acid-soluble fraction of retinae from chickens in the light or in the dark

Experiment	Time of exposure (h)	No. of pairs	Chickens in	Dark	Difference (%)	P
			c.p.m. gangliosides per mg protein			
			c.p.m. acid-soluble fraction per mg protein			
1	0.5	10	932 ± 100	1,384 ± 200	50.7 ± 12	< 0.01
2	2	14	3,160 ± 440	4,310 ± 560	35.7 ± 7	< 0.001
			c.p.m. sialic acid per nmol sialic acid			
			c.p.m. acid-soluble fraction per mg protein			
3	2	9	72 ± 6	83 ± 5	16.9 ± 4	< 0.01
1a	0.5	10	4,465 ± 360	3,993 ± 395	−8.0 ± 8	NS
2a	2	14	5,147 ± 538	4,681 ± 514	−5.1 ± 7	NS
			Chickens with one eye occluded			
			Exposed eye	Occluded eye		
			c.p.m. gangliosides per mg protein			
4	1	No. of chickens 18	141 ± 16	160 ± 15	18.5 ± 5	< 0.01

For experiments 1, 2 and 3, male Warren chickens were subjected, from hatching to the eighth day, to the conditions described previously¹, then maintained in the dark for 2 d and matched in pairs according to weight. After subcutaneous injections of 6-³H-glucosamine (specific activity 7.3 Ci mmol^{−1}; New England Nuclear, 0.7 µCi per g body weight), one animal of a pair remained in the dark while the other was exposed to 1,000 lx; chickens were decapitated 0.5 or 2 h later. For experiment 4, chickens were treated up to the tenth day as in the previous experiments; then they had one eye patched with black tape, injected with the radiochemical, exposed to light for 1 h and decapitated. Retinae were minced in water and gangliosides isolated by the trichloroacetic acid–phosphotungstic acid method⁷. For sialic acid determinations, gangliosides were hydrolysed in 0.1 N H₂SO₄ for 1 h at 80 °C and the hydrolysate was dialysed against water. Dialysed sialic acid was purified through Dowex 1 and determined by the thiobarbituric acid method⁸. Values given are means ± s.e.m.; the significance of the percentage differences was calculated by Student's *t* test for correlated data. Differences per cent are the means ± s.e.m. of individual differences for each matched pair. Values for retinae in light were considered 100%.

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Influence of antidiuretic hormone on release of lysosomal hydrolase at mucosal surface of epithelial cells from urinary bladder

THE permeability change induced by antidiuretic hormone (ADH) in amphibian urinary bladder is limited to the apical membrane of epithelial cells rich in intracellular secretion granules¹. These granules, which may correspond to lysosomes², are observed to migrate to and fuse with the apical membrane of toad bladder epithelia treated with oxytocin or cyclic AMP³. We have extended these observations and found that ADH elicits a marked, time-dependent increment in the release of lysosomal hydrolases into the extracellular medium of epithelial cells isolated from amphibian bladder. This effect is abolished by specific antiserum to ADH and reduced by preincubation of cells with cortisol. Additional experiments with intact bladder verify that the hydrolases so released occur predominantly at the mucosal surface. Moreover, cortisol antagonises the enhancement of transepithelial water transport and membrane conductance attributable to ADH action.

Epithelial cells were isolated from the urinary bladders of *Rana catesbeiana* (Mogul-Ed Corp., Oshkosh, Wisconsin) by procedures described previously^{4,5}. These cells were maintained at 22 °C and suspended in Ringer solution constituted as follows: 104.5 mM NaCl, 2 mM KCl, 1 mM CaCl₂, 1 mM MgSO₄, and buffered at pH 7.4 with 2.125 mM Na₂HPO₄/0.375 mM NaH₂PO₄. Aliquots (2 ml; approximately 3 × 10⁶ cells) were incubated in the presence or absence of hormone for selected times in a Dubnoff shaking incubator. The epithelial cells were then sedimented by centrifugation at 400g for 5 min at +15 °C in a Sorvall RC2-B centrifuge. Sedimented cells were solubilised in 0.1 N NaOH:0.1% (v/v) Triton X-100 (Rohm and Haas) at 37 °C (24 h) and analysed for protein by the method of Lowry *et al.*⁶. The cell-free incubation medium was centrifuged further for 1 h at 105,000g in a Beckman-Spinco Model L preparative ultracentrifuge. Activities of representative lysosomal enzymes were determined on aliquots of the resultant particle-free supernatant fractions. Total enzyme activity per min in each experiment was then expressed relative to mg epithelial cell protein.

Cathepsin B1 (EC 3.4.22.1) activity was determined essentially

as described by Szego⁷. Fluorescence of the reaction product of cathepsin-mediated hydrolysis of the substrate, carbobenzyloxy-Ala-Arg-Arg-4-methoxy-β-naphthylamide (provided by Dr R. E. Smith), was measured at the emission peak of 410 nm on an Aminco-Bowman spectrophotofluorometer, using an activation beam of 292 nm; 4-methoxy-β-naphthylamine standards (provided by Dr Edward Smithwick, Jr) were analysed concomitantly. β-Glucuronidase (β-D-glucuronide glucuronohydrolase, EC 3.2.1.31) activity was determined at pH 4.5 by the method of Fishman⁷ as modified by Musa *et al.*⁸, using the substrate, phenolphthalein β-D-monoglucosiduronic acid (Sigma) at 37 °C. Acid phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.2) activity was determined in the presence of 0.1 M sodium citrate buffer, pH 5.2, by the fluorometric method of Campbell and Moss⁹ as modified by Szego *et al.*¹⁰. Fluorescence of the product, α-naphthol, resulting from hydrolysis of the substrate, α-naphthylphosphate, was measured at the emission peak of 510 nm with an activation wavelength of 335 nm.

Epithelial cells in the several experiments were exposed to ADH (arginine vasopressin, Sigma), arginine vasopressin antiserum (provided by Drs A. A. Rosenbloom and D. A. Fisher), angiotensin II (Sigma), cortisol (free alcohol, Sharp and Dohme, West Point, Pennsylvania) or combinations of these agents. Cell viability in the presence and absence of these test reagents was evaluated by dye exclusion methods as described previously⁵. At least 95% of all cells used here excluded the dye, Trypan blue, at a final concentration of 0.05% in Ringer solution.

Determinations of activities of characteristic lysosomal enzymes released from epithelial cells incubated with and without ADH are presented in Table 1. Incubation in the presence of 1 mU ADH ml⁻¹ for 7.5 min resulted in statistically significant increases in the activities of acid phosphatase, β-glucuronidase and cathepsin B1, with mean increases of 66%, 24% and 167% over controls, respectively. Cells incubated with 1 mU ADH ml⁻¹ for 15 min responded in a similar manner. Acid phosphatase, β-glucuronidase and cathepsin activities rose by 37%, 15% and 133% over the values in controls. Although cells incubated with a higher concentration of hormone, 100 mU ADH ml⁻¹, for 15 min released a significantly greater activity of β-glucuronidase (*P* = 0.05), activities of the other lysosomal hydrolases did not vary significantly from those in the presence of 1 mU ADH ml⁻¹ (Table 1).

The effect of 1 mU ADH ml⁻¹ for 15 min in the presence of 50 p.p.m. antiserum specific for arginine vasopressin¹¹ was investigated. Control cells were incubated with antiserum alone. Activities of acid phosphatase and cathepsin in the incubation media of the hormone-treated groups were not significantly different from controls (Table 1). On the other hand, normal serum without hormone-specific antibody did not inhibit the

Table 1 Influence of ADH on lysosomal hydrolase activity in the extracellular medium of epithelial cells isolated from amphibian urinary bladder

Group	Incubation time (min)	Acid phosphatase activity (nmol per min per mg protein)	β-glucuronidase activity (nmol per min per mg protein)	Cathepsin B1 activity (nmol per min per mg protein)
Control	7.5	1.13 ± 0.03 (4)	3.04 ± 0.05 (5)	0.03 ± 0.01 (8)
ADH (1 mU ml ⁻¹)	7.5	1.87 ± 0.07 (4)*	3.77 ± 0.16 (5)†	0.08 ± 0.01 (8)*
Control	15	1.00 ± 0.10 (6)	3.19 ± 0.05 (5)	0.03 ± 0.01 (8)
ADH (1 mU ml ⁻¹)	15	1.37 ± 0.07 (5)†	3.67 ± 0.05 (3)*	0.07 ± 0.02 (8)‡
ADH (100 mU ml ⁻¹)	15	1.43 ± 0.07 (5)*	3.98 ± 0.10 (5)*	0.06 ± 0.00 (4)*
ADH antiserum (50 p.p.m.)	15	1.07 ± 0.07 (3)		0.03 ± 0.01 (3)
ADH antiserum and ADH (1 mU ml ⁻¹)	15	1.17 ± 0.03 (3)		0.03 ± 0.01 (3)

Activities of lysosomal enzymes are presented as mean ± s.e.m. with the number of determinations in parentheses. The significance of differences between hormone and control preparations was estimated by paired analysis with the Student's *t* test²⁴. In preliminary experiments, ADH was found to have no significant effect on enzyme activity *per se* in the assay conditions described in the text. In two additional experiments, 0.5 μg angiotensin II per ml, which is known to elicit a small hydro-osmotic effect in toad bladder²⁵, enhanced cathepsin activity to 110% of control cells incubated in the absence of the polypeptide for 15 min.

*Activity significantly different from control at *P* < 0.001.

†Activity significantly different from control at *P* < 0.01.

‡Activity significantly different from control at *P* < 0.05.

Table 2 Early mucosal release of cathepsin B1 elicited by serosal addition of ADH to intact bullfrog urinary bladder

Group	Incubation time (min)	Cathepsin B1 activity (nmol per min per mg dry weight)	
		Serosal side	Mucosal side
Control	7.5	0.03 ± 0.02 (8)	0.01 ± 0.00 (8)
ADH (100 mU ml ⁻¹)	7.5	0.03 ± 0.02 (8)	0.03 ± 0.00 (8)*
Control	15	0.03 ± 0.00 (7)	0.01 ± 0.00 (7)
ADH (100 mU ml ⁻¹)	15	0.03 ± 0.01 (7)	0.02 ± 0.00 (7)*

Intact bullfrog urinary bladders were incubated as sacs with Ringer solution in isosmotic conditions and otherwise as described before²⁶. The activity of cathepsin B1 was determined on aliquots of particle-free supernatant fractions collected from the mucosal and serosal media bathing the bladder sac. Total enzyme activity per min in each experiment was then expressed relative to mg dry weight of the bladder sac. All activities are presented as mean ± s.e.m. with the number of determinations in parentheses.

*Value significantly different from control at $P < 0.001$.

ADH effect (not shown). In the presence of ADH antiserum, therefore, the hormone does not induce increments of lysosomal enzyme activities in the extracellular medium.

The early influence of ADH on the differential release of cathepsin into the media bathing the serosal and mucosal sides of intact bullfrog urinary bladder is shown in Table 2. Incubation with 100 mU ADH per ml serosal solution for 7.5 min elicited a significant increment in cathepsin activity in the mucosal, but not in the serosal, medium. This result is consistent with the observations on isolated cells presented in Table 1 and further implies that the hormone-enhanced release of lysosomal hydrolases occurs predominantly at the apical surface of amphibian bladder.

In view of the known lysosome-stabilising action of cortisol¹², the effect of 100 mU ADH ml⁻¹ was tested for 7.5 min in isolated cells that had been preincubated with 3.3×10^{-6} M cortisol for 40 min. Cortisol alone had no significant effect on the extracellular activities of acid phosphatase (not shown; $n = 3$) and cathepsin (Table 3) as compared with paired controls incubated with the steroid vehicle, ethanol, at a final concentration of 0.1%. With addition of ADH following cortisol, activities of phosphatase and cathepsin, respectively, rose $19 \pm 10\%$ ($P > 0.20$, $n = 3$) and 67% over the values of controls exposed only to cortisol. These data reveal that membrane stabilisation by cortisol results in marked reduction of the effect of ADH on lysosomal enzyme release. Other experiments presented in Table 3 further indicate that cortisol acts to antagonise ADH-induced changes in the transport and electrical properties of bullfrog urinary bladder. Cortisol alone had no statistically significant influence on water permeability, short-circuit current or membrane conductance as compared with paired control hemi-bladders during a 40-min incubation period. The normal increment in water transport and membrane conductance in response to ADH¹³ was markedly reduced in bladders incubated with cortisol before exposure to ADH. The steroid, however, did not alter the effect of ADH on short-circuit current. These

data are consistent with previous reports which suggest that ADH has independent actions on sodium and non-electrolyte transport¹³.

Experiments in progress in our laboratory indicate that partially purified cathepsin B can elicit increments in lectin-mediated cellular agglutinability (for example, increase in membrane fluidity) at pH 7.4 (ref. 4). The action of ADH on non-electrolyte permeability in amphibian bladder is likewise considered to occur subsequent to the enhancement of membrane fluidity^{4,13,14}. The observed redistribution of membrane-associated particles on the apical surface of frog urinary bladder epithelium after oxytocin treatment is in accord with this hypothesis¹⁵. Since limited proteolysis at the membrane surface is known to alter cellular agglutinability^{16,17} and membrane fluidity¹⁸ in other cell types, the membrane action of the lysosomal protease, cathepsin B, should be investigated further.

The release of lysosomal enzymes into extracellular media or body fluids is thought to occur by a process of exocytosis following lysosomal membrane labilisation¹⁹. There is precedent for hormone-enhanced liberation of soluble proteins²⁰ and of lysosomal hydrolases^{10,21,22} into the particle-free medium of their specific target cell preparations. The potential physiological significance of these enzymes in hormone action has been reviewed^{2,4,23}. These data, taken in context with the present observations, suggest that ADH-enhanced mucosal release of membrane-bound hydrolases may contribute to the increments in membrane permeability and fluidity which characterise the hormone response at the apical membrane of epithelial cells rich in intracellular secretion granules.

Isolation of the two major epithelial cell populations from bullfrog bladder by centrifugation procedures and subsequent treatment of viable cells with ADH indicates that the hormone elicits lysosomal hydrolase release exclusively from "granular" cells and not from mitochondria-rich cells (Pietras, Naujokaitis and Szego, unpublished).

This study was aided by research grants from the US Public

Table 3 Counteractive influence of cortisol on transport and electrical properties of bullfrog urinary bladder and extracellular release of lysosomal hydrolase from isolated epithelial cells following addition of ADH

Group	Extracellular cathepsin B1 activity (nmol per min per mg protein)	Water permeability coefficient $\times 10^7$ (cm s ⁻¹)*	Membrane conductance $\times 10^4$ (mho cm ⁻²)	Short-circuit current (μ A cm ⁻²)
Control (0.1% ethanol)	0.03 ± 0.00 (3)	1100 ± 100 (4)	3.8 ± 0.2 (4)	31 ± 3 (4)
ADH (100 mU ml ⁻¹)	0.07 ± 0.00 (3)†	6400 ± 100 (4)†	4.6 ± 0.2 (4)†	48 ± 1 (4)†
Cortisol (3.3×10^{-6} M)	0.03 ± 0.00 (3)	950 ± 50 (4)	3.6 ± 0.3 (4)	30 ± 2 (4)
Cortisol and ADH	0.05 ± 0.01 (3)‡	3100 ± 300 (4)†	3.7 ± 0.2 (4)	47 ± 1 (4)†

Radioactive tracer and electrical techniques were used to study the transport of water and sodium, respectively, across bullfrog bladders mounted as flat sheets between two Lucite chambers as described previously^{13,14}. Transport and electrical measurements were made during the 60-min period following addition of ADH, cortisol or both hormones (cortisol being present for 40 min in mucosal and serosal media before serosal addition of ADH). Extracellular cathepsin B1 activity was determined as described in the text at 7.5 min following treatment of isolated epithelial cells with ADH, cortisol or both hormones. Ethanol, 0.1% final concentration, was present in all media.

*Values corrected as described before¹³ for the presence of unstirred layers (that is 160 μ m) in the bullfrog urinary bladder (R.J.P., unpublished).

†Significantly different from control value of group immediately above in table at $P < 0.001$.

‡Significantly different from control value of group immediately above in table at $P < 0.01$.

Health Service and the US National Science Foundation and by general research funds of the University of California.

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Action of various antidepressant treatments reduces reactivity of noradrenergic cyclic AMP-generating system in limbic forebrain

STUDIES on the pharmacology of the noradrenergic cyclic AMP-generating system in slices from the limbic forebrain of the rat¹ and on adaptive properties of this system in conditions of persistent changes in the availability of noradrenaline (NA) have revealed that the system may serve as a model for the central NA receptor in this area, and that its sensitivity to NA increases or decreases when the availability of NA at the receptor site decreases or increases respectively^{2,3}. Thus, hypersensitivity of the system has been achieved by treatment with reserpine², a drug known to

precipitate occasionally severe depressive reactions in man⁴, and the syndrome of which, when elicited in animals, is widely used as a model for depression^{5–7}. Conversely, the monoamine oxidase (MAO) inhibitors, pargyline and nialamide, caused a marked reduction in the reactivity of the cyclic AMP-generating system to NA after chronic administration⁸. To determine whether or not antidepressant drugs which do not elevate the level of monoamines in brain share the effect of MAO inhibitors on the noradrenergic cyclic AMP-generating system, we studied the effect of the tricyclic antidepressants, desipramine and iprindole, on the reactivity of the system to NA. Desipramine blocks the uptake of NA through the neuronal membrane^{8,9} whereas iprindole neither blocks the neuronal uptake of NA nor alters its metabolism or turnover^{10,11}, but is nevertheless a potent antidepressant^{12–14}. In addition, we have tested the effect of electroconvulsive treatment (ECT), as it is generally accepted to be one of the most effective treatments for severe depression¹⁵.

Male Sprague-Dawley rats (initial weight 180–220 g), housed four or five to a cage and kept in standard laboratory conditions (12-h light-dark cycle; food and water *ad libitum*) were used. The rats receiving antidepressant drugs were injected intraperitoneally with 10 mg kg⁻¹ d⁻¹ of desipramine or iprindole hydrochlorides for 4–8 weeks. Control rats received a corresponding volume of saline. Reserpine (Serpasil, CIBA-Geigy) was administered for 4 d at a dose of 2.5 mg kg⁻¹ d⁻¹. ECT was given by passing a current (100 mA; 300 ms) through ear clip electrodes. Controls had the electrodes attached, but no current was passed. Normal animals received ECT daily for 8 d, and the reserpine-treated animals received it for 4 d, approximately 2 h before each injection of reserpine.

The rats were decapitated at specified intervals after the last treatment, their limbic forebrain area (containing olfactory tubercles, septal and accumbens nuclei; anterior parts of the hippocampus and of the amygdaloid nuclei) was dissected as described previously², divided along the midline, chopped into blocks of approximately 0.5 mm³ and incubated at 37 °C following slightly modified² procedures of Kakiuchi and Rall¹⁶ and Palmer *et al.*¹⁷. After a 30-min preincubation period and change of the incubation medium (NaCl 118 mM; KCl 5 mM; CaCl₂ 2.5 mM; KH₂PO₄ 2 mM; MgSO₄ 2 mM; NaHCO₃ 24 mM; EDTA-Na 0.02 mM; glucose 10 mM; O₂-CO₂ 95:5; pH 7.4), the slices were incubated for an additional 14 min. At this time, slices from one half of the forebrain were exposed to a specified concentration of NA, whereas those from the corresponding

Table 1 Effect of antidepressant drugs and ECT on response to NA of cyclic AMP-generating system in limbic forebrain of rat

	Duration of treatment (d)	Time of death* (h)	NA concentration (μM)	Cyclic AMP response to NA† (pmol per mg protein ± s.e.m.)
Saline	28–56	24	5	23.9 ± 2.5 (12)
Desipramine (10 mg kg ⁻¹)	28–56	24	5	6.6 ± 2.0 (14)¶
Iprindole (10 mg kg ⁻¹)	28–56	24	5	7.2 ± 2.4 (16)¶
Handling	8	18	10	57.9 ± 1.9 (20)
ECT	8	18	10	40.3 ± 3.7 (20)‡
Handling	8	42	10	88.4 ± 6.2 (5)
ECT	8	42	10	44.4 ± 7.8 (5)¶
Handling	4	18	50	177.3 ± 14.1 (6)
Handling + reserpine (2.5 mg kg ⁻¹)	4	18	50	268.2 ± 17.6 (7)§
ECT + reserpine (2.5 mg kg ⁻¹)	4	18	50	208.8 ± 17.2 (7)¶

*Time after last injection of drug, ECT or handling.

†Difference of levels of cyclic AMP between preparation exposed to NA and non-exposed preparation from corresponding half of the limbic forebrain. Numbers in parentheses denote number of pairs. Basal levels of cyclic AMP were not changed by the antidepressants or ECT (17.8 ± 2.6 pmol per mg protein in saline-treated and 35.1 ± 1.4 pmol per mg protein in handled group).

‡*P* < 0.05.

§*P* < 0.01.

¶*P* < 0.001 (difference between control and experimental; drug and/or ECT group).

||Not significantly different from control (handled group), but different (*P* < 0.05) from handled, reserpine-treated group.

half served for the determination of the basal level of cyclic AMP. The reaction was terminated 10 min later by homogenisation of the tissue slices in 3.5 ml 0.3 N HClO₄; a 0.5-ml aliquot of the homogenate was used for the determination of the protein concentration by the method of Lowry¹⁸. Cyclic AMP was isolated by ion-exchange chromatography and assayed by the method of Gilman¹⁹. All values were corrected for recoveries and cyclic AMP was expressed in pmol per mg protein. Student's two-tailed *t* test was used for the statistical evaluation of the data.

The effect of chronic treatment with tricyclic antidepressants and of ECT is shown in Table 1. Prolonged treatment with antidepressant drugs reduced the responses of the cyclic AMP-generating system by approximately 70%. A much shorter treatment with ECT reduced the response by 30% in rats killed 18 h after the last shock, and, interestingly, by 50% in rats killed a day later. Reserpine treatment (2.5 mg kg⁻¹ daily for 4 d) enhanced the response to NA by approximately 50%, confirming earlier results². Concomitant ECT practically abolished the increased cyclic AMP response to NA (Table 1). The response to NA has not been significantly changed in preparations from rats receiving tricyclic antidepressants for a period of 2 weeks or less. Our results complement data obtained with MAO inhibitors, which depressed the cyclic AMP response to NA by 50–60% after prolonged but not short term treatment³ and reveal a common mode of action at post-synaptic noradrenergic receptor sites for antidepressants regardless of their action at presynaptic sites, and for ECT. The decreased reactivity to NA after ECT may provide the biochemical basis for the antagonism by ECT of stimulant effects induced by combined treatment with desipramine and Ro4-1284 (ref. 20) or amphetamine²¹, and for the potentiation by ECT of the reserpine syndrome and α -methyltyrosine-induced catalepsy²¹.

Our data emphasise the dissociation of acute biochemical effects of antidepressant drugs from their therapeutic action, which occurs only after chronic treatment for weeks. The demonstrated decrease in noradrenergic receptor function as a consequence of various antidepressant treatments—pharmacotherapy and ECT—should focus future neurochemical research on the elucidation of molecular mechanisms of altered aminergic receptor function and provide a new theoretical framework for studies on both pathogenesis and therapy of affective disorders.

We thank USV Pharmaceutical Corporation and Wyeth Laboratories for desipramine and iprindole, respectively. Our studies were supported by the US Public Health Service.

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Tubulin in postsynaptic junctional lattice

TUBULIN, the primary protein of microtubules^{1,2}, has been identified as a major soluble protein in isolated synaptosomes^{3,4}. Indirect evidence for the presence of tubulin in the synaptosomal plasma membranes themselves has been accumulating for some time. Colchicine-binding activity, used as an assay for the presence of tubulin^{1,2}, has been found in synaptosomal plasma membranes^{5–7}. These membranes, prepared by several different techniques, all show a prominent protein band of apparent molecular weight 52,000–53,000 when solubilised and electrophoresed in sodium dodecyl sulphate–polyacrylamide gel systems^{8–12}; such gel techniques applied to well characterised microtubule preparations yield molecular weights for tubulin which have been reported to range from 52,000 to 56,000 (refs 1 and 2), the precise value depending on the particular

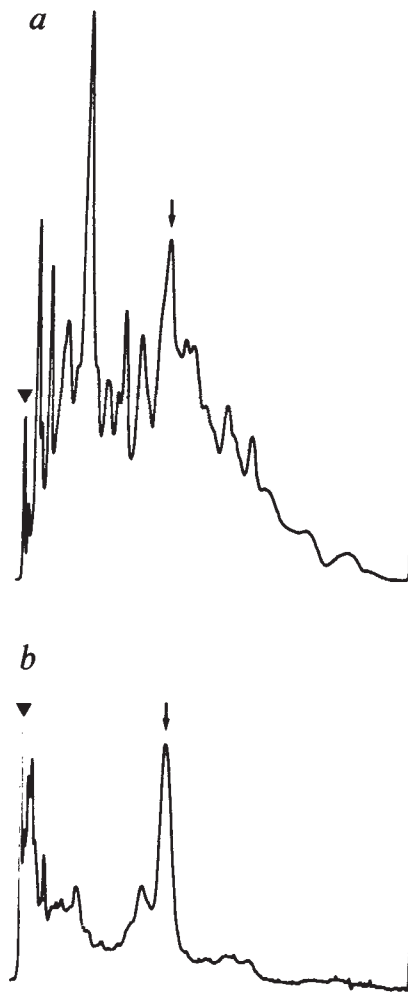


Fig. 1 Densitometer scans of the proteins of synaptosomal plasma membranes (a) and isolated postsynaptic junctional lattices (b), stained with Coomassie brilliant blue after electrophoresis in 7.5% polyacrylamide gels with a continuous buffer system of 0.1% (w/v) sodium dodecyl sulphate, 0.1 M Tris-HCl, pH 7.4. The sample buffer contained dodecyl sulphate–protein 2:1 (w:w) and 0.2% 2-mercaptoethanol in 5mM Tris-HCl, pH 7.4. Triangles indicate the tops of the gels, and the arrows indicate the bands with an apparent molecular weight of 50,300.

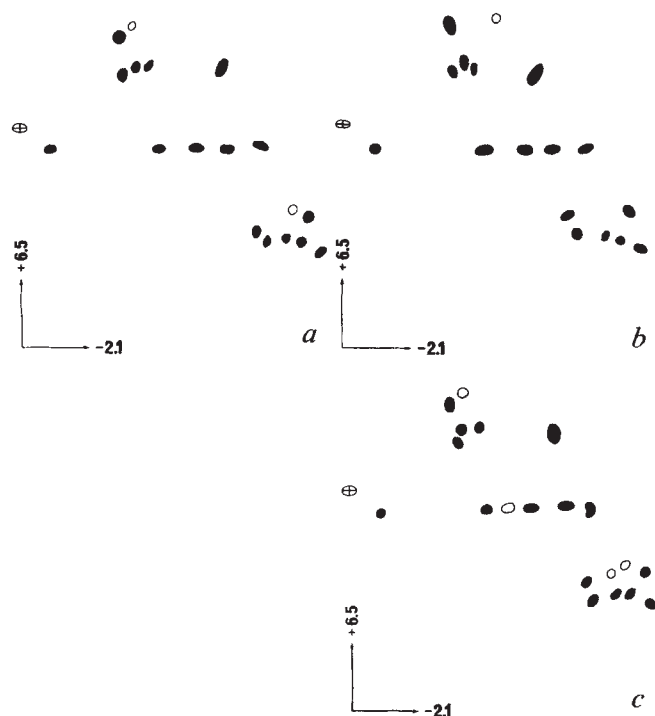


Fig. 2 Tracings of autoradiographs of the tryptic maps obtained from proteins labelled with ^{125}I after electrophoresis in sodium dodecylsulphate-polyacrylamide gels¹⁸. Crossed circles mark the origins, solid circles the peptides common to all three maps and open circles the remainder. The starting proteins were: *a*, the 50,300-dalton protein from synaptosomal plasma membranes; *b*, the 50,300-dalton protein from isolated postsynaptic junctional lattices; *c*, electrophoretically pure tubulin.

electrophoretic system used. Sequential solubilisation of synaptosomal plasma membranes with Triton X-1000 and N-lauroyl sarcosinate yields isolated postsynaptic densities¹³ whose major protein has a molecular weight of 53,000 (ref. 14), and similarly digestion of the synaptosomal lipid unit membrane by sodium deoxycholate results in isolated postsynaptic junctional lattices¹⁵ with a major protein of approximate molecular weight 53,000 (ref. 12). We report here the identification of tubulin in the postsynaptic junctional lattices of rat forebrain both by two-dimensional electrophoresis of ^{125}I -labelled tryptic peptides and by electron microscope immunohistochemistry.

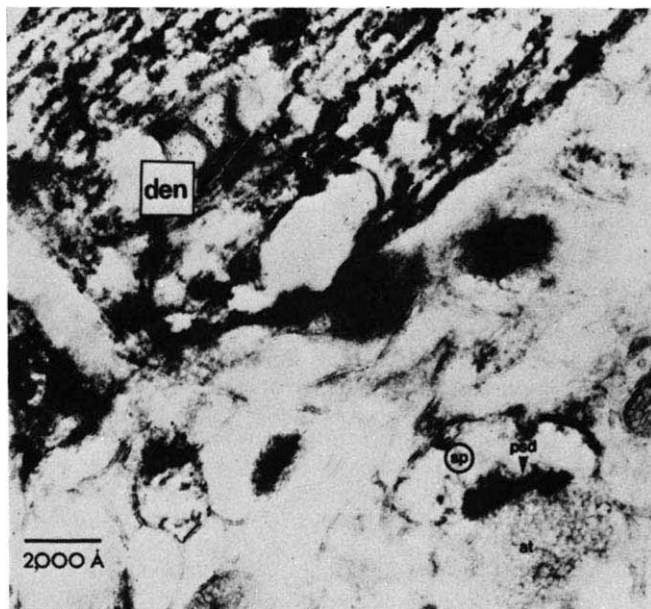
Synaptosomal plasma membranes were prepared from rat forebrain by the method of Jones and Matus¹⁶, with the slight modifications that all sucrose solutions and the lysis buffer contained 50 μM calcium chloride and the sucrose solutions were also buffered with 5 mM HEPES, pH 7.4. Postsynaptic junctional lattices were isolated by incubating the membranes at a final protein concentration of 1.7 mg ml⁻¹ in 10% (w/v) sodium deoxycholate, 1 mM phenylmethylsulfonylfluoride, and 50 μM calcium chloride for 2 h at 37 °C, followed by dilution with a two-thirds volume of distilled, deionised water and pelleting at 100,000g for 1 h at 4 °C (see ref. 12). Of the membrane protein, 98% is solubilised by this procedure. Figure 1 shows densitometer scans of the proteins of the starting synaptosomal plasma membranes and the isolated postsynaptic lattices, stained with Coomassie brilliant blue after electrophoresis in sodium dodecyl sulphate-polyacrylamide gels. The band in each gel indicated by the arrow has an apparent molecular weight of 50,300 and comigrates with added rat brain tubulin prepared by the repeated polymerisation method of Shelanski *et al.*¹⁷. These bands were excised from the gels, particular care being taken to omit the shoulder (molecular weight 54,300) on the membrane

peak. The tubulin band from a similar gel of electrophoretically pure tubulin, itself prepared by elution from polyacrylamide gels of once repolymerised tubulin, was also excised. The proteins from these slices were eluted, concentrated, iodinated by a chloramine T reaction, performic acid oxidised and then digested with trypsin and the resultant peptides purified, using the methods of Bray and Brownlee¹⁸. Samples of these peptides were electrophoresed at 3,000 V in 2 dimensions on Whatman 3 MM paper, first for 75 min in pH 6.5 buffer (10% pyridine, 0.3% acetic acid) and then for 75 min in pH 2.1 buffer (8% acetic acid, 2% formic acid). The electrophoretograms were exposed to X-ray film for 42–168 h. Figure 2 shows the autoradiographic maps of the tyrosine-containing tryptic peptides of the 50,300-dalton protein from synaptosomal plasma membranes, the 50,300-dalton protein from postsynaptic junctional lattices, and tubulin. The number of spots in the tubulin map (20) agrees well with the expected number of tubulin tyrosine peptides (18–19) (ref. 19) and the number of iodinated peptides previously reported (18–20) (ref. 18). The three tryptic fingerprints are almost identical, and thus the three proteins from which they were derived are strikingly homologous.

Approximately 300 μg of tubulin which had been excised from polyacrylamide gels of once-repolymerised rat brain tubules, eluted, and concentrated, was intramuscularly injected into a rabbit in 50% Freund's complete adjuvant and comparable booster shots administered at 10-d intervals. This technique has been used to produce monospecific antibodies to actin²⁰ and myosin²¹. Figure 3 is an electron micrograph immunohistochemically illustrating the cellular localisation of the rat brain tubulin antigen in rat cerebral cortex²². The peroxidase reaction product is clearly visible on dendritic microtubules and is also present at the postsynaptic lattice of the visible axodendritic synaptic junction. In all the material we have examined, incubation of brain tissue with anti-tubulin serum in the immunoperoxidase procedure produced staining only in association with microtubules and the postsynaptic junctional lattices²³.

We have thus established that the major postsynaptic

Fig. 3 Electron micrograph of a rat cerebral cortex slice which was incubated with rabbit anti-tubulin serum, washed with phosphate buffered saline (PBS), incubated with peroxidase-labelled sheep anti-rabbit gamma globulin serum, washed again with phosphate-buffered saline, and the immunohistochemical staining developed by 30 min exposure to a solution of 5 mg 3,3'-diaminobenzidine in 10 ml of PBS, pH 7.2, to which had been added 10 μl of hydrogen peroxide.



junctional lattice protein and tubulin share a common antigen and are so closely similar in primary structure that their tryptic digests are virtually identical. These results confirm the earlier tentative identification of tubulin as the major component of the postsynaptic density, where it probably has an important structural role in providing a matrix for more specialised proteins of functional importance in synaptic transmission^{12,14}. Some form of functional role for the tubulin itself cannot, however, be discounted at present. It has been proposed that "linkage elements" associated with the cell membrane and pharmacologically similar to tubulin are involved in the selective cellular control of particular membrane components²⁴. Although such elements may be present throughout the synaptosomal plasma membrane, more of these "tubulin-like" molecules would be expected in specialised areas, especially ordered arrays such as are present at the synaptic junction¹⁵. The observed distribution of tubulin in the synaptosomal plasma membrane and postsynaptic junctional lattice precisely matches the hypothesis that tubulin functions as a membrane-associated molecular linkage element.

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Post-transcription control of isocitrate lyase induction in the eukaryotic alga *Chlorella fusca*

WE have shown previously¹ that poly(A)-containing RNA can be isolated from the green alga *Chlorella fusca* 8p (ref. 2) and that this RNA has messenger-like properties, as it is polydisperse and stimulates polypeptide synthesis *in vitro* in a wheat embryo system. We show here that the *Chlorella* protein isocitrate lyase has been identified as a product of *in vitro* translation of poly(A)-containing RNA, so confirming messenger function in this fraction. We have used the *in vitro* translation system as a means of assaying for the presence of isocitrate lyase message sequences in poly(A)-containing RNA isolated from *Chlorella* cultured under different conditions, and we report here that slow-growing non-induced cells may contain high levels of mRNA for this enzyme although the enzyme is not synthesised.

Isocitrate lyase is an adaptive enzyme^{3,4}, and its synthesis

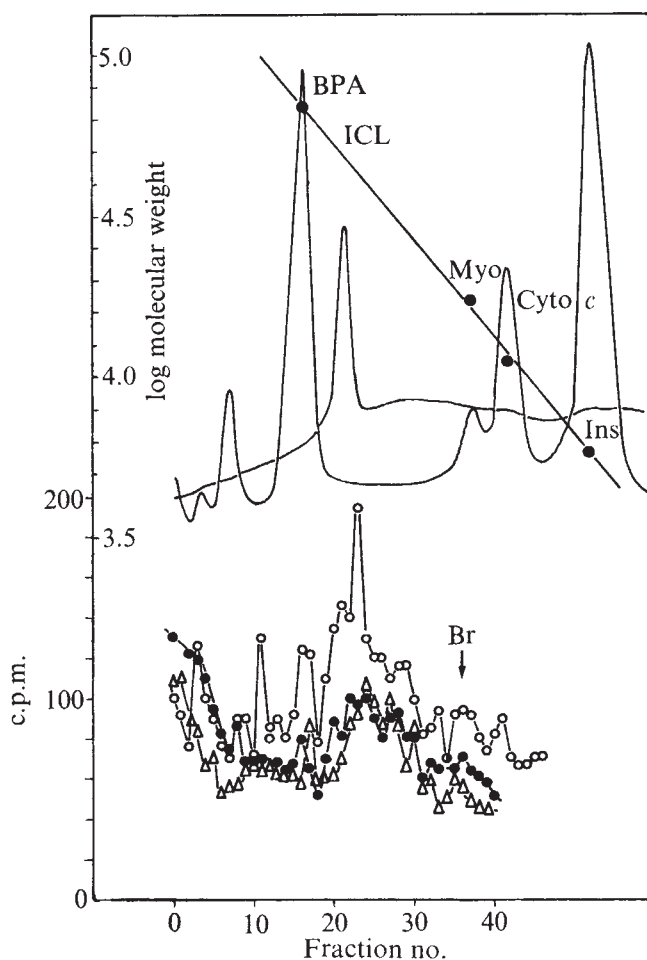


Fig. 1 SDS gel electrophoresis of immunoprecipitated protein produced by *in vitro* protein synthesis, in a wheat embryo system. Total *Chlorella* RNA was separated on a cellulose column into an unadsorbed fraction and an adsorbed fraction (described fully in ref. 1). The adsorbed fraction was poly(A)-containing RNA of which 46 µg were added to one cell-free protein synthesis assay (○). The unadsorbed fraction from the column was mostly rRNA of which 50 µg were added to a second cell-free protein synthesis assay (△). A control assay where no RNA was added was also performed (●). *Chlorella* RNA was isolated as described previously¹, from a culture containing 2.5×10^7 cells ml⁻¹ which had been incubated in the dark with 0.1% sodium acetate for 90 min (acetate-adapting *Chlorella*). The cell-free protein synthesis mixtures were prepared by the method of Roberts and Paterson¹⁰ as described previously¹, but scaled up to a total volume of 400 µl, and were incubated at 30 °C for 2 h. The radioactive amino acid included was ³⁵S-methionine (10 µCi, 200 Ci mmol⁻¹). After incubation all assays were treated as follows: 5 µg ovalbumin and 50 µl anti-ovalbumin serum were added and precipitates formed after 2 h at room temperature were removed by centrifugation (preprecipitation, see text). Supernatants were mixed with carrier antigen (5 µl pure isocitrate lyase, 0.75 mg ml⁻¹) and 25 µl of specific anti-isocitrate lyase serum. After 16 h at 4 °C the immune precipitate was recovered by centrifugation, washed three times with 1 ml of 5 mM Tris-acetate, pH 7.5, dissociated with 20 µl of 0.2 M NaOH for 15 min at room temperature and neutralised by addition of 20 µl of 0.2 M HCl and 60 µl of 8 M urea. The resulting mixtures were made 1% in sodium dodecyl sulphate (SDS) and 0.01 M in 2-mercaptoethanol and incubated at 100 °C for 2 min before electrophoresis in 7.5% polyacrylamide gels¹¹. After electrophoresis the gels were sliced into 1-mm segments and the radioactivity in each slice was determined as previously described¹. Br, Bromophenol blue marker. The upper part of the figure shows absorbance at 650 nm of two gels run under identical conditions and loaded with pure isocitrate lyase (ICL) or insulin (Ins), cytochrome c (Cyto c), myoglobin (Myo) and bovine plasma albumin (BPA). Both gels were stained with Coomassie brilliant blue.

is induced in *Chlorella* when acetate is added to darkened cultures. There is temporal restriction of isocitrate lyase synthesis in synchronous *Chlorella* cells³ and its synthesis is prevented when photosynthesis is possible⁶ or when glucose is present in the medium^{3,4}, indicating a catabolite repression control mechanism in addition to the inducing effect of acetate. When fully induced, isocitrate lyase is an unusually abundant enzyme constituting 7% of total soluble protein⁷. This suggested that isocitrate lyase mRNA might be a relatively abundant RNA species.

Preliminary experiments showed that rabbit antiserum which was raised against isocitrate lyase purified to homogeneity⁸, precipitated less than 0.05% of the radioactivity in an extract from an autotrophic *Chlorella* culture grown in the presence of ³⁵S-sulphate⁹, when the antiserum was added in the presence of sufficient unlabelled pure isocitrate lyase to enable immune precipitation. When unlabelled pure isocitrate lyase was added to wheat embryo cell-free protein synthesis assays and precipitated with specific antiserum, the immune precipitate was contaminated with proteins resulting from nonspecific adsorptions. In the experiment described under Fig. 1 this nonspecific effect was reduced by a preprecipitation step, where products of cell-free protein synthesis assays were mixed with ovalbumin which was precipitated with a specific anti-ovalbumin serum.

Figure 1 shows the results of electrophoretic analysis of the proteins precipitated from cell-free protein synthesis assays by anti-isocitrate lyase serum. The addition to the cell-free system of poly(A)-containing RNA isolated from *Chlorella* cultures in the process of isocitrate lyase synthesis, resulted in synthesis of a protein *in vitro*, which was absent from assays which had received either no added RNA, or *Chlorella* rRNA. The peak of radioactive protein had the same electrophoretic mobility as pure isocitrate lyase, which is also shown in Fig. 1. Isocitrate lyase is a tetrameric protein and under the conditions used to dissolve the immune precipitate, it was completely dissociated into subunits of molecular weight ~ 47,000.

To minimise the problem of nonspecific adsorption of proteins to the antibody, the anti-isocitrate lyase serum was immobilised by cyanogen bromide linkage to Sepharose beads (anti-ICL-Sepharose). When pure isocitrate lyase was adsorbed by a column of anti-ICL-Sepharose, only a small proportion of the enzyme was displaced by 0.01 M NaOH whereas the remainder was displaced quantitatively with 0.1 M NaOH (Fig. 2). Figure 2 also shows the elution of radioactive protein from an anti-ICL-Sepharose column which had been loaded with the products of a cell-free protein synthesis assay, primed with poly(A)-containing RNA isolated from *Chlorella* in the process of isocitrate lyase synthesis. Gel electrophoresis of protein eluted by 0.1 M NaOH is shown in Fig. 3, where it can be seen that a profile of peaks closely resembling those obtained with pure isocitrate lyase was obtained. Preparation of samples for electrophoresis as performed in this experiment did not completely dissociate isocitrate lyase and a distribution of monomers and multimers was obtained. It is evident that protein adsorbed to the anti-ICL-Sepharose column and

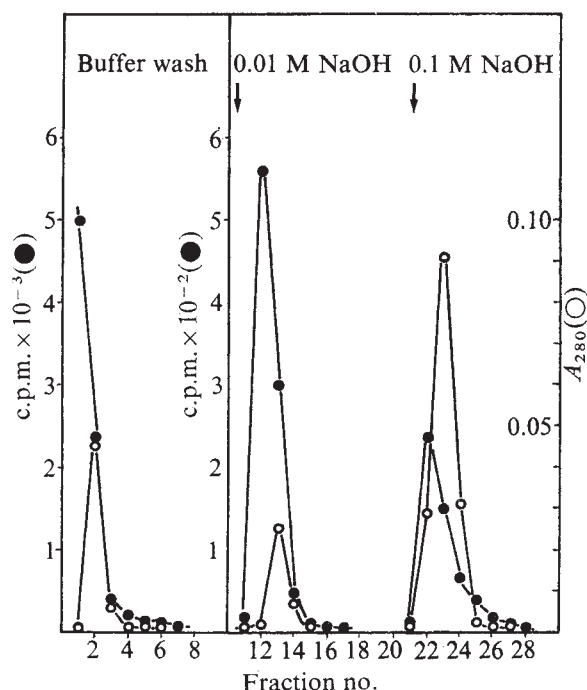


Fig. 2 Elution of products of *in vitro* protein synthesis from a column of anti-isocitrate lyase serum covalently linked to sepharose (anti-ICL-Sepharose). The data are for products of *in vitro* translation of poly(A)-containing RNA from acetate-adapting *Chlorella*, in a 200 μ l cell-free mixture incubated at 30 °C for 3.5 h, but otherwise as described under Fig. 1, (●). Pure isocitrate lyase was also adsorbed to a column and eluted (○). To prepare anti-ICL-Sepharose, 2 ml of anti-isocitrate lyase serum (15 mg ml⁻¹ protein) were linked to 1 g of cyanogen bromide-activated Sepharose 4B following the manufacturer's instructions (Pharmacia). Anti-ICL-Sepharose, suspended in 0.5 M NaCl, 0.005 M sodium phosphate, pH 7.0 was packed in 1-ml columns and washed with 5 ml of 0.005 M sodium phosphate, pH 7.0 (phosphate buffer) before use. Products of cell-free protein synthesis assays or pure isocitrate lyase (40 μ g) were applied to the column and allowed to adsorb for 1 h at room temperature, before washing through with 20 ml of phosphate buffer (buffer wash). Elution with 2 ml of 0.01 M NaOH was followed by a further 20 ml of phosphate buffer (0.01 M NaOH eluate). Lastly 2 ml of 0.1 M NaOH were applied and this also was followed with 20 ml of phosphate buffer (0.1 M NaOH eluate). Hot trichloroacetic acid-insoluble radioactivity in each fraction was counted as previously described¹. Elution of pure isocitrate lyase was followed by measurement of absorbance at 280 nm.

which was only displaced by the higher NaOH concentration with largely isocitrate lyase. This, in turn, demonstrated the presence of message sequences for isocitrate lyase in poly(A)-containing RNA from *Chlorella*.

In the conditions of batch culture which we used⁸, autotrophic *Chlorella* cultures grew exponentially until cell number density reached about 10⁷ cells ml⁻¹, then growth rate declined as they became more and more light limited, finally ceasing at a density of about 5 × 10⁷ cells ml⁻¹. At no stage of growth does isocitrate lyase synthesis occur

Table 1 Fractionation of *in vitro* translation products of *Chlorella* poly(A)-containing RNA on anti-ICL-Sepharose columns

Source of poly(A)-containing RNA	Buffer wash	Hot TCA-insoluble radioactivity (c.p.m.)		% of total c.p.m. in 0.1 M NaOH fraction
		0.01 M NaOH eluate	0.1 M NaOH eluate	
(1) Acetate-adapting cells	103,600	25,000	13,500	9.4
(2) Control (minus RNA)	50,000	530	1,900	3.6
Exponential autotrophic cells	50,900	5,400	1,600	3.0
Light-limited autotrophic cells	44,100	2,800	5,800	12.3

Data assembled from Fig. 2 (1) and Fig. 4 (2). Pooled fractions were sampled and hot trichloroacetic acid-insoluble radioactivity measured. For (1) cell-free translation mixtures were incubated for 3.5 h at 30 °C. For (2) cell-free translation mixtures were incubated for 2 h at 30 °C.

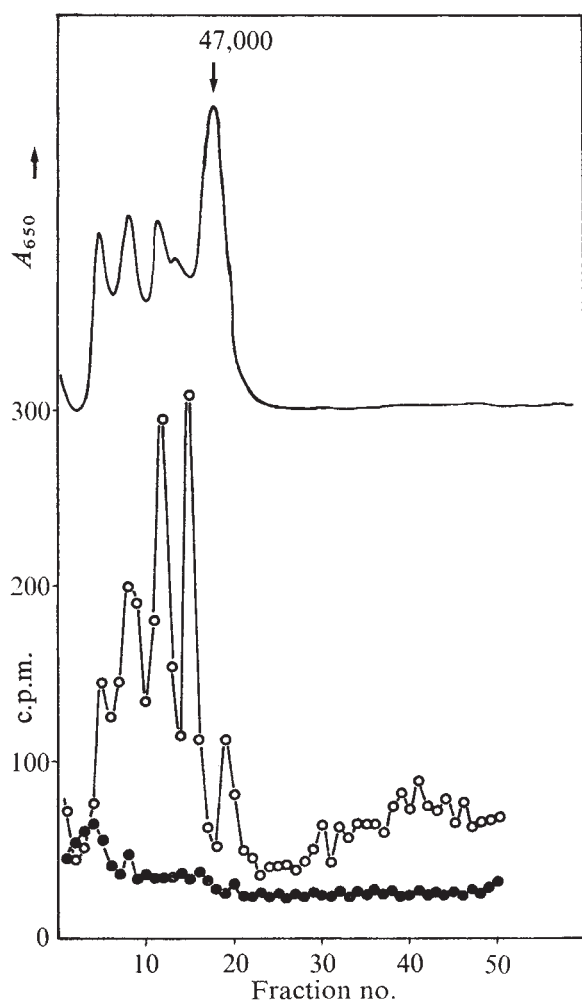


Fig. 3 SDS disc gel electrophoresis of *in vitro* translation products eluted with 0.1 M NaOH from anti-ICL-Sepharose columns (see Fig. 2). *In vitro* translation of poly(A)-containing RNA from acetate-adapting *Chlorella* is described in Fig. 1 and fractionation of the translation products on anti-ICL-Sepharose is shown in Fig. 2. Protein in the 0.1 M NaOH eluate was subjected to electrophoresis (○). Products of a control *in vitro* translation assay (minus RNA) were also fractionated (Fig. 4) and protein in the 0.1 M NaOH eluate subjected to electrophoresis (●). Both samples were prepared for electrophoresis as follows: pooled column fractions were made 5% in trichloroacetic acid; precipitated protein was recovered by centrifugation, washed twice with 5 ml of ethanol, resuspended in gel buffer¹² containing 1% SDS and 0.01 M 2-mercaptoethanol and heated to 100 °C for 2 min. Electrophoresis was carried out in 15% SDS gels¹² after which they were cut into 1-mm slices which were incubated overnight at 37 °C in a toluene-based scintillation mixture including 5% Protosol (New England Nuclear) and counted for radioactivity. The upper part of the figure shows absorbance at 650 nm of a gel which had been run with 20 µg of pure isocitrate lyase and stained with Coomassie brilliant blue.

unless acetate is the sole available carbon and energy source.

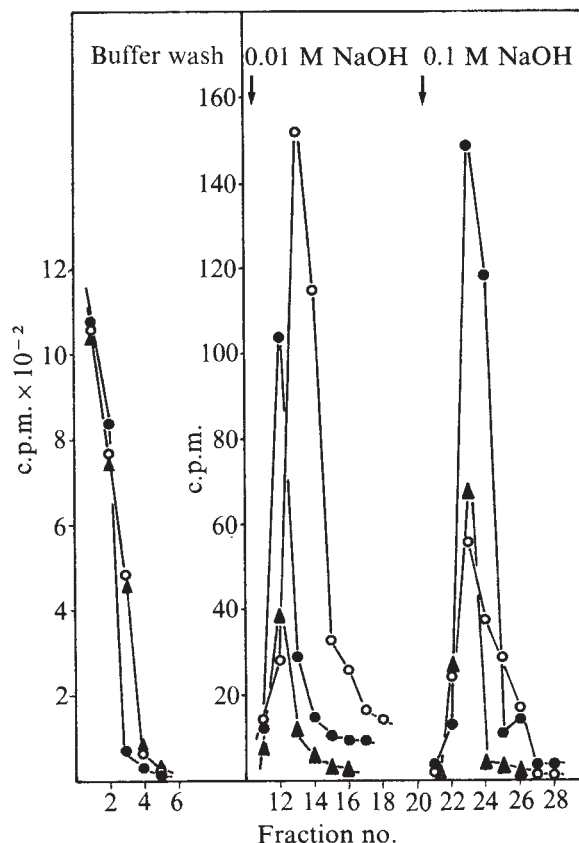
Poly(A)-containing RNA was isolated both from exponentially growing autotrophic cultures and from light-limited cultures. Analysis of products of *in vitro* protein synthesis directed by these RNA samples, using anti-ICL-Sepharose columns, is shown in Fig. 4. When poly(A)-containing RNA from exponentially growing cells was tested, the amount of radioactive protein in the 0.1 M NaOH eluate from the column was the same as the control, in which the cell-free protein synthesis assay was performed without added RNA (see also Table 1). In contrast, addition to the cell-free system of poly(A)-containing RNA isolated from light-limited autotrophic cells resulted in isocitrate lyase synthesis as demonstrated by the larger amount of

radioactive protein in the 0.1 M NaOH eluate (Fig. 4 and Table 1). In a separate experiment (results not shown) this protein was found to have the same electrophoretic mobility as pure isocitrate lyase (analogous to Fig. 3).

The absence of detectable isocitrate lyase mRNA in exponentially-growing, photosynthesising cells and its presence in cells adaptively synthesising isocitrate lyase, provides evidence for the operation of a transcriptional control, but it is also firmly demonstrated that a post-transcriptional control can operate, since messenger accumulates without being translated, as low light limits the growth rate.

When *Chlorella* cells use acetate as their sole carbon source, the activity of the glyoxylate cycle is necessary for the synthesis of intermediary metabolites which are normally more readily produced by photosynthesis or the metabolism of glucose¹³. The synthesis of isocitrate lyase correlates with the detection of glyoxylate cycle activity in this strain of *Chlorella*³ and the present study demonstrates that the adaptive synthesis of the enzyme in rapidly growing cells follows the accumulation of mRNA from previously undetectable levels. This evidence provides further support for the conclusion that synthesis of the enzyme in rapidly growing cells is initiated by transcription, which was previously drawn tentatively from the effect of inhibiting functional RNA synthesis⁴. There is therefore a parallel with the control of gene expression in prokaryotic cells¹⁴⁻¹⁶.

Fig. 4 Elution from anti-ICL-Sepharose of *in vitro* translation products of poly(A)-containing RNA extracted from autotrophically grown *Chlorella*. Poly(A)-containing RNA was isolated¹ from an autotrophic *Chlorella* culture collected during exponential growth (cell number density, 6.5×10^6 cells ml⁻¹), (○) and from a culture which had become light-limited (cell number density, 1.44×10^7 cells ml⁻¹) (●). These poly(A)-containing RNA samples were translated in the wheat embryo cell-free system in a final volume of 200 µl, but otherwise as described in Fig. 1. Translation products were fractionated on anti-ICL-Sepharose columns as described in Fig. 2. Products of a control cell-free assay to which *Chlorella* RNA had not been added, were also fractionated (▲). Hot trichloroacetic acid-insoluble radioactivity was measured.



The direct estimation of isocitrate lyase mRNA, however, provides evidence for at least one additional control point acting at the post-transcriptional level, which is more specific in its requirements for release of enzyme synthesis. In light-limited cells the supply of intermediary metabolites is sub-optimal and the accumulation of isocitrate lyase mRNA is initiated, but this is not sufficient to cause synthesis of the enzyme. Therefore the restriction of isocitrate lyase synthesis to conditions when acetate is present to act as substrate for the glyoxylate cycle and with no alternative carbon source available, is achieved by the operation of post-transcriptional control. This control may be lacking in the thermophilic strain of *Chlorella*, in which darkening without addition of acetate is sufficient to promote maximum synthesis of isocitrate lyase¹⁷.

It is possible that the mRNA which is accumulated, but not translated, in light-limited cells is held at a stage requiring further processing, such as specific cleavage from a large nuclear precursor (HnRNA), or export from the nucleus. This interpretation supposes that the wheat embryo *in vitro* protein-synthesising system can translate unprocessed messenger, as can *Xenopus* oocytes¹⁸. There is evidence, however, that post-transcriptional control is exerted when mRNA is fully processed and is already supporting isocitrate lyase synthesis. Glucose, which makes the glyoxylate cycle redundant, causes an immediate cessation of enzyme synthesis^{4,6}, in contrast to the gradual cessation of synthesis which follows the use of an inhibitor to prevent further transcription⁴. Many metabolite levels in *Chlorella* are altered by changes in carbon source^{19,20} and these metabolites could operate post-transcriptional control by modifying the binding of a regulator protein to mRNA^{21,22}, or by modifying the nascent protein to produce either abortive folding²³ or enhanced sensitivity to protease attack²⁴.

Whatever the precise mechanism of post-transcriptional control for isocitrate lyase synthesis, the existence of this control in eukaryotic algae, taken together with evidence for the operation of similar mechanisms in mammalian cells²⁵, suggests that post-transcriptional controls may be common in eukaryotes.

P.C.L.J. thanks the Science Research Council for support.

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Is Fanconi's anaemia defective in a process essential to the repair of DNA cross links?

FANCONI'S anaemia (FA) is a rare autosomal recessive disease of man, characterised by a progressive hypoplastic pancytopenia associated with diverse congenital anomalies^{1,2}, spontaneous chromosome breakage³ and predisposition to leukaemia and other cancers^{4,5}. Lymphocytes from FA patients were found to be excessively susceptible to chromosome breakage by di- or polyfunctional alkylating agents^{6,7}, and this was interpreted as a possible indication of defective DNA repair of the FA cells⁷. Seeking evidence for this, I have tested lymphocytes from FA patients for their chromosome response to mono- and difunctional mitomycins in relation to the cell cycle phase at the time of treatment, and have found that FA cells have a specific defect in the repair of pre-aberration lesions induced by difunctional mitomycin; the lesions are possibly DNA cross links of the interstrand type. The rationale of this experiment was the knowledge that if damage to DNA is left unrepaired it can be linked causally to the formation of chromosome aberrations by a process that resides in semiconservative DNA replication⁸⁻¹². Since the repair of DNA damage is a rate-limiting process, the treatment of repair-proficient cells in early G₁ would leave more time for repair before the damage is fixed into chromosome aberrations during the S phase, and consequently would result in fewer chromosome aberrations than treatment during the transition from G₁ to S. In the repair-deficient cells, however, DNA damage induced by treatment in any position of G₁ would be linked maximally to the formation of chromosome aberrations.

Heparinised venous blood was obtained from two male patients with FA aged 6 and 7 yr (identified as FA7 and FA9) and a normal healthy person as a control. The procedure has been described before¹⁰. Briefly, a series of whole-blood microcultures was set up using basal medium NCTC-109 supplemented with 20% foetal calf serum and 3% phytohaemagglutinin, and fixed for chromosome preparation after 70-74 h of incubation. At various times before fixation, cells were treated for 30 min with difunctionally reacting mitomycin C (MMC) or its monofunctional derivative, decarbamoyl mitomycin C (DCMMC, a gift from Dr K. Nakano, Kyowa Hakko Kogyo Co.) in conjunction with ³H-thymidine, and incubation was continued for a corresponding recovery time in culture medium free of test compounds. The preparations were processed for autoradiography and analysed for their metaphase labelling pattern and the presence of chromosome aberrations in labelled and unlabelled mitoses.

The changes in metaphase labelling index are shown in Fig. 1. Even in the absence of mitomycins, FA cells passed more slowly than normal through S and G₂. Moreover, in contrast to normal cells, most FA cells collected at 70-74 h were still in their first mitosis, as indicated by a comparatively small amount of the second maximum metaphase labelling index. Treatment with mitomycins reduced the rate of progression through the cell cycle, and the effect was more marked when the FA cells were treated with MMC. Figure 2 shows that ³H-thymidine alone had little effect on chromosomes of normal or FA cells. Although DCMMC was less efficient than MMC, both induced significant chromosome aberrations of the chromatid type. The

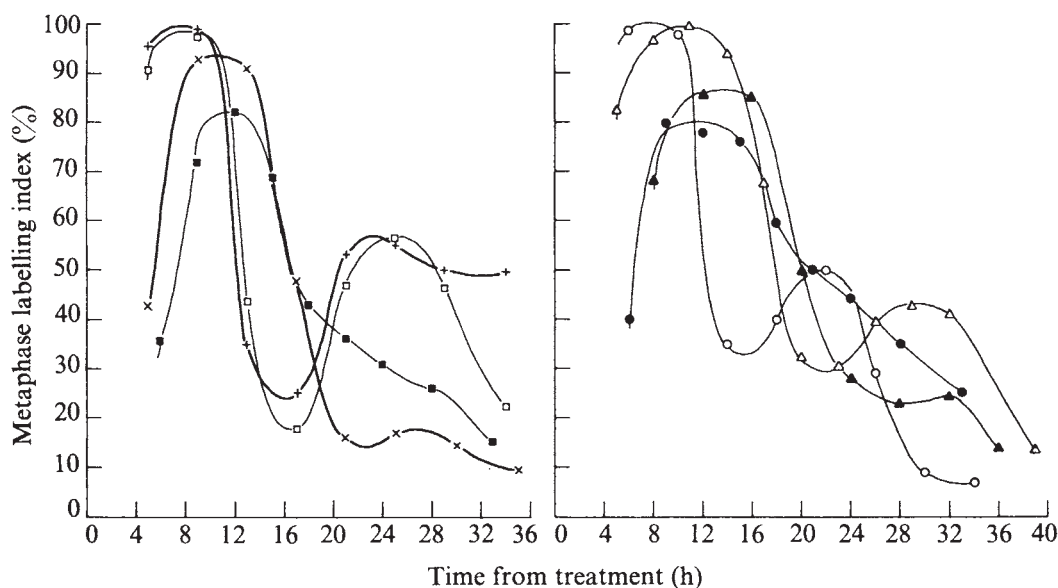


Fig. 1 Changes in the proportion of labelled mitoses as a function of time from treatment. At various times before fixation, cells were treated for 30 min at 37 °C with DCMMC or MMC which was made up in medium TC-199 containing ^3H -thymidine ($1 \mu\text{Ci ml}^{-1}$) or with ^3H -thymidine alone, washed and re-incubated for the indicated recovery time in the medium NCTC-109 supplemented with 20% foetal calf serum and $5 \times 10^{-5} \text{ M}$ cold thymidine. All cultures were fixed at any time between 70 and 74 h of total incubation time with a 2-h prefixation treatment with colchicine. Chromosome preparations were made according to the standard air-drying technique using hypotonic treatment in

0.9% sodium citrate before fixation in methanol-acetic acid (3:1). Preparations were processed for autoradiography using Kodak NTB3 emulsion. Metaphase labelling index was determined on 250–400 mitoses. +, Normal cells treated with ^3H -thymidine alone; ×, FA9 cells treated with ^3H -thymidine alone; □, normal cells treated with DCMMC ($20 \mu\text{g ml}^{-1}$); ■, FA7 cells treated with DCMMC ($50 \mu\text{g ml}^{-1}$); ○, normal cells treated with MMC ($0.5 \mu\text{g ml}^{-1}$); △, normal cells treated with MMC ($1.5 \mu\text{g ml}^{-1}$); ●, FA7 cells treated with MMC ($0.1 \mu\text{g ml}^{-1}$); ▲, FA9 cells treated with MMC ($0.1 \mu\text{g ml}^{-1}$).

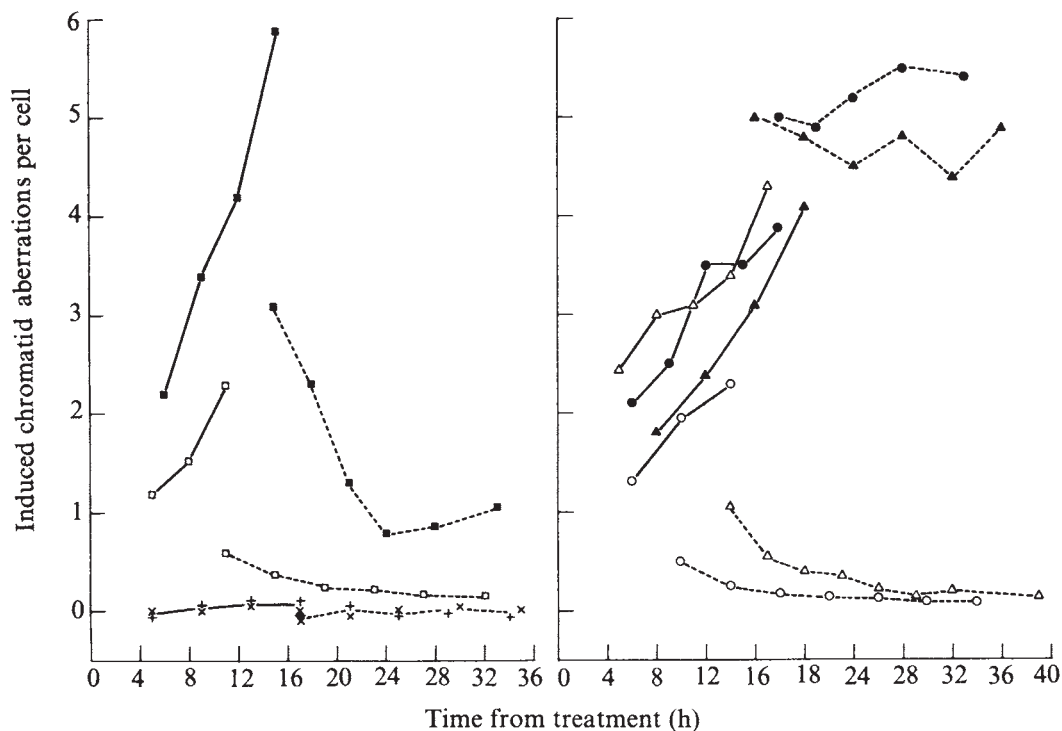


Fig. 2 Changes in the frequency of chromatid aberrations in labelled (solid lines) and unlabelled (dashed lines) mitoses as a function of time after treatment. After recording of the labelling pattern and location of mitoses, the silver grains were removed and chromatid aberrations were scored in the preselected mitoses (100 for each point). Chromatid aberrations scored are gaps, breaks and exchanges occurring at the chromatid level. The scoring does not include the cells in S phase at the time of

treatment and recovered at their second mitosis. The frequencies of chromatid aberrations are expressed as the net amount of induced aberrations, that is, observed frequencies minus spontaneous levels, which were 0.07, 2.00 and 2.14 per normal, FA7 and FA9 cell, respectively, as determined in their 72-h cultures, which were kept untreated during incubation. The symbols used are the same as in Fig. 1.

patterns of the chromosome response of normal cells to DCMMC and MMC and that of FA cells to DCMMC were as predicted for repair-proficient cells. With respect to formation of chromosome aberrations, the S phase, more particularly its very beginning, was the most sensitive stage. The chromosome aberrations were less frequent in unlabelled mitoses that were in the pre-DNA-synthesis stage at the time of treatment, and their frequencies varied with the recovery time, being maximum in late G₁, decreasing with increasing recovery time. When tested with MMC, however, FA cells were most sensitive at G₁ and, moreover, yielded a constant rate of aberrations when treated in various parts of G₁, and a decreasing yield as the S phase progressed. This pattern of chromosome response to MMC is comparable with that of xeroderma pigmentosum cells to 4-nitroquinoline-1-oxide¹⁰. These cells have an impaired capacity to repair DNA alterations induced by ultraviolet light and some chemicals, including 4-nitroquinoline-1-oxide^{13,14}. These results indicate that in the FA cells pre-aberration lesions induced by DCMMC can be repaired, but those induced by MMC at any time before DNA synthesis are not repaired and link maximally to the chromosome aberrations formed during the subsequent S phase. Since the FA cells are also abnormally susceptible to chromosome breakage by the psoralen-plus-light reaction⁷, which is highly specific to native DNA and induced interstrand cross links¹⁵, it is probable that the MMC-induced lesions that are irreparable in the FA cells are DNA cross links of the interstrand type.

In bacteria, repair of cross links is controlled by *uvr* and *recA* genes, and it has been suggested to be mediated by a sequential process involving excision of one cross linked arm and a subsequent recombination of homologous duplexes^{16,17}. When the excision repair capacity of FA lymphocytes was tested, their level of DCMMC- and MMC-stimulated unscheduled DNA synthesis was comparable with that of normal cells. Thus if a repair mechanism like that proposed for cross links in bacteria can apply to human cells, the FA cells could be defective in the later step of the sequential repair process. A simple application of this repair process, especially an excision step by endonucleolytic incision cannot, however, easily explain the repair of cross links in mammalian cells.

With respect to differing sensitivity of cell stage to chromosome breakage, xeroderma pigmentosum cells respond to MMC as the repair-proficient cells while they are deficient in unscheduled DNA synthesis induced by MMC (my unpublished data). Similarly, rat kangaroo cells show a chromosome response of the repair-proficient type to sulphur mustard¹² in spite of their unresponsiveness, in terms of unscheduled DNA synthesis, to ultraviolet light¹⁸. Furthermore, cross links induced by sulphur mustard can be repaired in mouse L cells¹⁹ which have very little ability to excise ultraviolet damage²⁰. These observations suggest that, unlike bacteria in which sensitivity to MMC, sulphur mustard and ultraviolet light is commonly associated with the *uvr* gene^{21,22}, mammalian cells can repair cross links by a process independent of the excision ability responsible for the repair of damage induced by ultraviolet light. The identification of the site of the primary defect in FA cells awaits elucidation of such a repair process. My results, however, strongly suggest that FA cells are defective in a biological system which constitutes an essential step in the repair of DNA interstrand cross links.

I thank Dr A. Ozawa for his cooperation. This work was supported by grants from the Ministry of Education and the Ministry of Health and Welfare of Japan.

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Organisation and evolution of *Drosophila virilis* heterochromatin

CENTRIC heterochromatin in *Drosophila* metaphase chromosomes is organised into large blocks which show various degrees of condensation in prometaphase¹ and various degrees of decondensation when living cells are Hoechst treated². By sequential staining with the fluorescent dyes Hoechst and quinacrine, three different types of blocks are distinguishable in *D. virilis*. Each block seems to contain only one DNA satellite and similar staining blocks, along with their respective satellite, appear or disappear as units during speciation; this was first suggested from a study of *D. hydei* sibling species¹ and here confirmed for the *D. virilis* group.

The *virilis* group species in Fig. 1 are phenotypically almost indistinguishable and interspecific crosses, excepting with *D. ezoana*, produce fertile offspring³. Maps of their polytenised euchromatin are identical except for inversions and translocations^{3,4}. Heterochromatin differs drastically within the group as evidenced by acrocentric fusions, heterochromatin elimination and the presence or absence of various satellite DNAs in centromeric heterochromatin (Fig. 1).

Sequential staining of *D. virilis* metaphase chromosomes with Hoechst, quinacrine and Giemsa reveals three different types of heterochromatin blocks enabling one to identify each chromosome by the blocks it possesses (Fig. 1). The remaining species lack H⁺ or Q⁺ blocks so their chromosome arms, being of similar size, cannot usually be identified. In interspecific hybrids, however, such as the *D. virilis* × *D. texana* hybrid of Fig. 2, the euchromatic arms of *D. texana* chromosomes are often somatically paired with homologous and identifiable arms of *D. virilis* chromosomes. Here, by comparing the fluorochromatically differentiated heterochromatin of homologous arm pairs from different species, one sees that *D. texana* has 65% less heterochromatin than *D. virilis* and lacks block α₁. The *virilis* group's heterochromatin is thus organised into distinct blocks and similar blocks are lost or gained during speciation (Fig. 1).

In *D. virilis*, there seems to be a 1:1 relationship between the three different fluorochromatic blocks and the three different DNA satellites which is based on indirect evidence from various sources. *D. melanogaster* shows five satellites which are located in centric heterochromatin⁵⁻⁷. Some of these satellites, as localised by *in situ* hybridisation, are confined to only one or a few chromosomes⁷. Four satellites are homogeneous non-interspersed tandem repeats of a small oligonucleotide; the repeats are at least 600 kilobases (kb) long⁷. *D. virilis* satellites present a similar story. ³H-RNA complementary to satellite I hybridised *in situ* to heterochromatin of all chromosomes⁸; however, since heterologous satellite hybrids have melting points close to the *in situ* annealing conditions used⁹ and autoradiographic resolution is limiting, the exact location of satellite I was not determined. Sarcosyl lysates of *D. virilis* cells, having

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² Gmyrek, D., and Syllm-Rapoport, I., *Zeitschr. Kinderheilk.*, **91**, 297-337 (1964).

³ Schroeder, T. M., Anschutz, F., and Knopp, A., *Humangenetik*, **1**, 194-196 (1964).

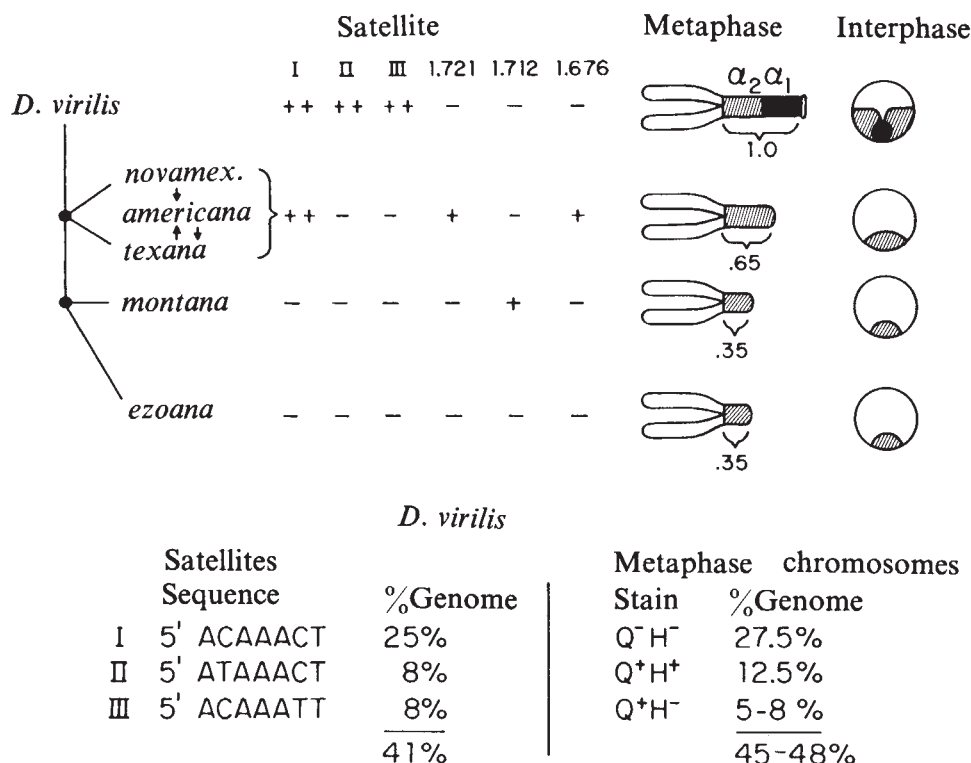


Fig. 1 Phylogenetic relations of the *D. virilis* group^{3,4} are presented as family tree. Species which evolved from *D. montana* and *D. ezoana* are not included. Satellites common to each species^{10,11} are indicated. Minor satellites, which comprise less than 5% of the genome, are designated by their buoyant density in neutral CsCl. One stylised chromosome arm and an interphase nucleus from female larval neuroblasts of each species are presented. Alpha heterochromatin of each *D. virilis* chromosome is composed of two fluorochromatic blocks. The distal α_2 block is Q⁻H⁻ while the proximal α_1 block is Q⁺H⁺ for chromosomes X, 2 and 4, Q⁺H⁻ for chromosome 3, and Q⁻H⁻ for chromosome 5 (ref. 1). Chromosomes from the remaining five species show less heterochromatin and no Q⁺ or H⁺ blocks of heterochromatin. All six species have the same amount of euchromatin³; the amount of heterochromatin is expressed as prometaphase chromosome length of heterochromatin/length euchromatin where the amount of euchromatin is normalised to 1.0. For example, *D. americana* and *D. texana* each have an average of 0.65 units of heterochromatin associated with each euchromatic chromosome arm. *D. novamexicana* has approximately the same amount of heterochromatin as these two previous species even though most of it is associated with one chromosome pair. Centric heterochromatin aggregates into a chromocentre in diploid (right hand column) and polytene interphase nuclei. *D. virilis* interphase nuclei are particularly interesting because the heterochromatic chromocentre is divided into three regions possibly representing the three different satellites²⁴. The central region is H⁺ and partially separates the two Hoechst-dull regions. The other *virilis* group species lack H⁺ or Q⁺ material in their chromosomes and interphase nuclei. The size of their interphase chromocentres parallels their content of metaphase heterochromatin. The table shows the three heptanucleotide sequences which, when tandemly repeated, constitute the three *D. virilis* satellites^{10,11}. The percentage of the diploid genome each satellite comprises is shown next to its base sequence. The percentage prometaphase chromosome length devoted to each permutation of Hoechst-quinacrine brightness is from previous data¹ and is arranged to correspond to the satellite data. Estimates of percentage genome devoted to a particular 'type' of chromatin are rather inexact because of differential chromatin condensation during various stages of mitosis.

DNA molecules averaging at least 90 kb in length, show excellent satellite separation after analytical buoyant density ultracentrifugation^{8,10,11}. Thus, similar to the findings with *D. melanogaster* satellites, one heptanucleotide is repeated many times without interdigitation and forms almost pure repeating heptanucleotide tracts at least 90–180 kb long. By extending these molecular weight findings a little, one can imagine a pure repeating heptanucleotide tract 15,000 kb long. This is just enough DNA to fill one fluorochromatic block (one-quarter of a chromosome) (Fig. 3). Each block, being homogeneous in its DNA, would show a homogeneous fluorescence after quinacrine or Hoechst staining and this is indeed seen¹. There are three satellites in heterochromatin and three differently staining blocks of heterochromatin. The amount of each satellite and amount of heterochromatin showing a particular quinacrine-Hoechst permutation can be arranged to correspond quite well (Fig. 1). This correspondence ascribes satellite I to the Q⁻H⁻ blocks. Satellite I, having the highest GC content of the three satellites, would be predicted to correspond to the most weakly fluorescing blocks¹²⁻¹⁴ as it does. Satellites II and III are absent from *D. americana*, *D. texana* and *D. novamexicana*^{10,11} as are their ascribed Q⁺H⁺ and Q⁺H⁻ blocks (Figs 1 and 2). In hybrid cells containing somatically paired chromosomes, this loss seems to be a simple excision of the Q⁺H⁺ or/and Q⁺H⁻ block (Fig. 2). When comparing the sibling species *D. hydei*, *D. neohydei* and *D. eohydei*, the same relationships hold^{1,15}. Here

the presence of fluorochrome bright and dull blocks parallel the presence of AT-rich and GC-rich satellites, respectively. Here again the polytene banding patterns are almost identical, only the non-polytenised centric heterochromatin is different between species. The lack of Q⁺ or H⁺ material in *D. americana*, *D. texana* and *D. novamexicana* cannot be due to lack of a specific protein which confers fluorochrome-bright properties to heterochromatin because the staining properties of each chromosome in *virilis* × sibling species hybrids remains unchanged. Finally, the absence of satellite I in *D. ezoana* and *D. montana* is paralleled by an extreme loss of Q⁻H⁻ heterochromatin. The simplest model of chromosome organisation in accord with all existing data is that each fluorescent block of centric heterochromatin in *D. virilis* contains one continuous repeating DNA molecule consisting of a single heptanucleotide repeated about two million times which, when coated with protein, condensed and packaged, forms a homogeneous appearing block of centric heterochromatin with fluorescent properties determined by the heptamer it contains (Fig. 3).

In chromosomes of the *virilis* group, certain alterations of heterochromatin occurred, were selected for and subsequently fixed during evolution. Knowing these alterations, we can now make some statements concerning the organisation and evolution of heterochromatin in *Drosophila*.

(1) Heterochromatin is more taxonomically divergent than external morphological characteristics^{1,15}; one can more easily

identify the fly by looking at its metaphase chromosomes than by looking at the fly itself. Speciation has proceeded with the loss or gain of large heterochromatin blocks (Fig. 1) although the euchromatic arms have remained relatively unchanged^{3,4}.

(2) The 2-3 metacentric in *D. texana* (Fig. 2) and a similar X-4 metacentric in *D. americana* were thought to represent simple Robertsonian fusions of two *D. virilis* acrocentrics^{10,11} but this is obviously not true (Fig. 2). If these metacentrics actually evolved from the present *D. virilis* chromosomes, then fusion was preceded by, or concomitant with, loss of α_1 blocks, loss of the short Q^+H^- right arms and relocation of the *virilis* centromeres into Q^-H^- heterochromatin. Although the exact process of chromosome fusion that actually took place remains unknown, it was irreversible and more complex than the simple, reversible Robertsonian fusions which occur between telocentric chromosomes during speciation in mice.

(3) Satellite DNA cannot account for all the heterochromatin in any of the *virilis* group species (Fig. 1). This is most evident in *D. ezoana* which lacks satellite but, like all *virilis* group species, has some heterochromatin in each chromosome. The complete absence of satellite DNA in many of these species^{10,11}, even in alkaline CsCl gradients, argues against the presence of a cryptic satellite. Thus, each Q^-H^- block in *D. virilis*, *americana*, *texana* and *novamexicana* must be composed of two DNAs, satellite I and non-satellite, and two different Q^-H^- blocks which cannot be distinguished by fluorometric means. The non-

satellite block would correspond to the heterochromatin remaining *D. ezoana* and *D. montana*. Being ubiquitous within the group, this block must contain the heterochromatic genes and about the euchromatin-heterochromatin junction (Fig. 3). The heterochromatin of the *D. melanogaster* X is similarly Q^-H^- (ref. 1), lacks satellite DNA⁷ and contains the sex-linked heterochromatic genes¹⁶.

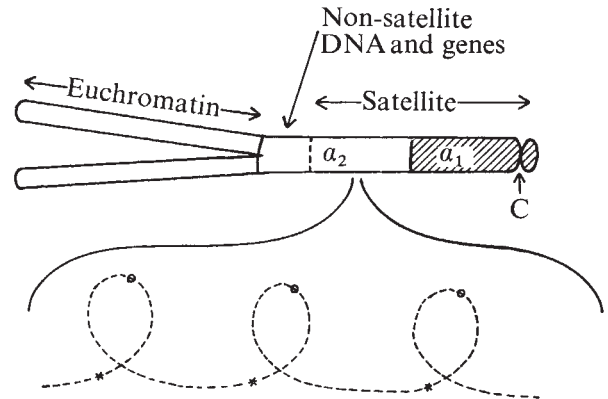


Fig. 3 The single DNA molecule, which runs the length of the chromosome²⁰, shows three successive levels of DNA organisation in *D. virilis* heterochromatin. The basic level is a tandemly repeated heptanucleotide (—)^{10,11}. The second level, a 2-30+ repeating unit, was detected in *D. melanogaster*^{17,18} as equally spaced restriction endonuclease sensitive sites (*, ○) similar to bovine satellite I DNA¹⁹. Lastly, the 2-30-kb repeats are packaged into large fluorochromatic blocks which contain about 17,000 kb = one-quarter of a chromosome. The α_1 block and the short arm opposite the centromere (C) are fluorochrome bright and contains satellites II or III. The dull α_2 block contains satellite I in its proximal region and non-satellite DNA in its distal region. Although Peacock *et al.*⁶ reported satellite DNA in euchromatic polytene bands 21CD of *D. melanogaster*, I find this hybrid to have a 15 °C lower melting point than centromeric hybrids. Thus, 21CD DNA differs from centromeric satellite⁹ and euchromatic regions may indeed be free of true centromeric satellite DNA.

(4) Similar blocks of heterochromatin and similar satellites appear or disappear during speciation even though these similar blocks or satellites are usually on many different chromosomes. This may imply selection for interchromosomal interaction of like blocks of heterochromatin. In interphase nuclei, the heterochromatin aggregates into a chromocentre wherein similar blocks of heterochromatin aggregate on to themselves to the exclusion of others (Fig. 1). This aggregation also seems to direct somatic non-disjunction in colcemid-anaphase nuclei of *D. virilis*⁵. There are three types of chromosome-chromosome pairing in *Drosophila* somatic nuclei: somatic pairing of identical euchromatin, centric aggregation of identical or similar centric heterochromatin and ectopic pairing of polytene bands which are often morphologically¹⁷ or chemically^{18,19} similar. All three pairings involve two chromosome segments which contain identical or suspiciously similar DNA. Selection for interchromosomal pairing of heterochromatin would explain the simultaneous appearance or disappearance of quasi-dispersed satellites during speciation.

I thank Dr David E. Comings for support and review, and Drs B. J. Conner and T. A. Okada for review.

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Received May 22; accepted August 20, 1975.

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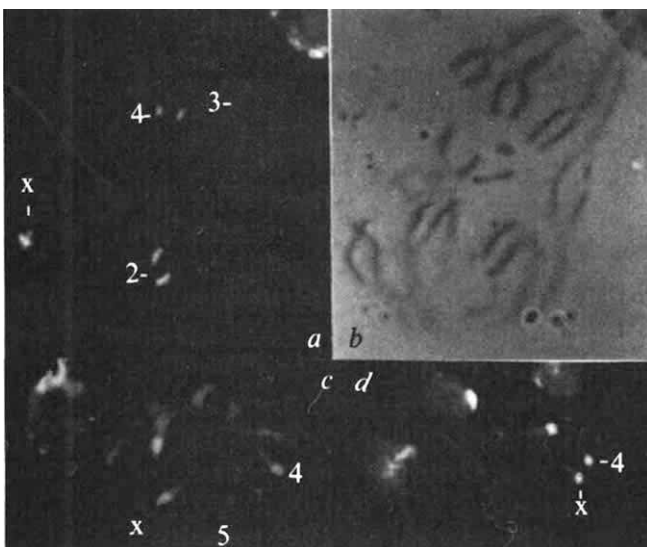


Fig. 2 Neuroblast prometaphase chromosomes from hybrid *D. virilis* × *D. texana* third instar larvae sequentially stained with Hoechst, quinacrine and Giemsa to distinguish each chromosome. Neuroblast metaphase preparations were stained with Hoechst¹, destained (three overnight rinses in pyridine), stained with quinacrine and finally stained with Giemsa. The pyridine removal method enables one to initially scan a Hoechst-stained slide when chromosomes are brightest and subsequently stain with the dimmer (quinacrine) fluorochrome. *a*, *c* and *d*, Hoechst stained; *b*, Giemsa stained from *a*. *D. virilis* chromosomes are indicated by number. Somatic pairing of homologous euchromatic arms allows one to unambiguously identify each *D. texana* arm because the homologous *D. virilis* arm is identifiable. For example, in (*a*), the upper arm of the 2-3 metacentric of *D. texana* pairs with the Q^+H^- *D. virilis* chromosome 3. The lower arm pairs with the Q^+H^+ *D. virilis* chromosome 2. Even though it is difficult to distinguish between the two Q^+H^+ chromosomes 2 and 4 in *D. virilis*, one can easily do this in such hybrids where *D. virilis* 2 must pair with one arm of the *D. texana* 2-3 metacentric. This sibling species-somatic pairing method should be quite useful for chromosome identification in many other dipteran groups. Alpha heterochromatin condenses earlier than euchromatin^{1,8} and is most apparent in Giemsa stained preparations of very early prometaphase (*b*). The Q^+H^+ blocks of α_1 heterochromatin condense even earlier than α_2 heterochromatin¹ so the H^+ blocks in early prometaphase (*a*) appear relatively smaller than the H^+ blocks in late prometaphase (*c* and *d*). *D. texana* chromosomes look like *D. virilis* chromosomes which have lost the α_1 block of heterochromatin.

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Segregation of RD-114 and FeLV-related sequences in crosses between domestic cat and leopard cat

TYPE C viruses of the RD-114 (ref. 1) group have been isolated, either spontaneously or after chemical induction, from cell cultures of the domestic cat (*Felis catus*)^{2–4}. Nucleic acid sequences related to the RD-114 genome are in the DNA of all domestic cats^{5–8}. Thus these viral genomes are transmitted vertically from parent to offspring as integral components of cat cellular DNA. Although the family Felidae consists of closely related animals, only four *Felis* species have been found to contain RD-114-related sequences. These include the domestic cat, the European wildcat (*F. sylvestris*), the sand cat (*F. margarita*), and the jungle cat (*F. chaus*); other members of the Felidae lack nucleic acid sequences related to RD-114 (ref. 9). The observation that RD-114 is partially related to the endogenous baboon type C viruses^{10–12} and that sequences related to RD-114 are found in the cellular DNA of all Old World monkeys led to the postulate that this group of viruses originated from an endogenous primate type C virus¹³ transmitted horizontally to the germ line of ancestors of certain *Felis* species during the Pliocene or early Pleistocene somewhere in the region of the Mediterranean basin⁹.

A second distinct group of type C viruses, the feline leukaemia viruses (FeLV), also has been isolated from domestic cats¹⁴. Although FeLVs are horizontally transmitted among domestic cats, genes partially related to the RNA genome of FeLV are found in *F. catus* DNA¹⁵ and in the DNA of the other species of Felidae which contain RD-114 related nucleic acid sequences¹⁶. Viruses of the FeLV group are also postulated to have been transmitted to an ancestor of these *Felis* species, but to have originated from a rodent rather than a primate source¹⁶.

The leopard cat (*F. bengalensis*) is a spotted wildcat found throughout South-east Asia which lacks RD-114 and FeLV-related DNA sequences (RD[–], FL[–])⁹. Since leopard cats produce viable offspring when bred with domestic cats (RD⁺, FL⁺), we studied the segregation of both sets of virogenes in F₁ hybrids and in the progeny of a backcross to the RD[–], FL[–] parent. The cellular DNA of the F₁ hybrids contains half the number of copies of each set of sequences. The RD and FL virogenes segregate together in the backcrossed animals in a manner consistent with their localisation at a single chromosomal site.

The reassociation kinetics obtained by hybridising ³H-DNA transcripts of viral RNA to cellular DNA can be used to estimate relative gene frequencies by determination of half C₀t values (the midpoint of the renaturation curve)¹⁷. The number of gene copies can also be estimated by plotting reassociation kinetics as the reciprocal of the fraction of unhybridised ³H-DNA against C₀t (Wetmur–Davidson plot)¹⁸. In such plots, the slope is proportional to the number of copies of those sequences measured. Using RD-114 ³H-DNA probes,

multiple copies of virus-related sequences can be detected in the cellular DNA of stray domestic cats, domestic cats reared in a germ-free environment (Merck, Sharp and Dohme, West Point, Pennsylvania) and in European wildcats (*F. sylvestris*) (Fig. 1a and Table 1). The C₀t_{1/2} values ranged from 120 to 170. In contrast, the cellular DNA of the leopard cat completely lacks RD-114 related sequences. The C₀t_{1/2} values for the self-annealing of non-repetitive domestic cat cellular DNA, and for the hybridisation of the ³H-DNA RD-114 probe to the DNA of a canine thymus cell line infected with RD-114, ranged from 1,800 to 2,000 (Fig. 1, Table 1). Given that C₀t_{1/2} values of 1,800–2,000 are obtained with genes present in a single copy per haploid genome, domestic cat and European wildcat cellular DNAs contain 10–13 copies of RD-114-related sequences per haploid genome. Since these copies represent a family of diverging gene sequences only partially related to one another¹⁹, the calculated number of copies may be an underestimate^{20, 21}.

Leopard cat males were mated to domestic cat females and the F₁ hybrids studied. These DNAs contain a complete complement of sequences related to the RD-114 probe, but only half the number of copies present in the domestic cat parent (Fig. 1a). The C₀t_{1/2} values (275–350) represent, as a minimum estimate, five to seven virogene copies per haploid genome. Two kittens obtained from an F₁ hybrid female backcrossed to the leopard cat (Fig. 2) were also studied. Figure 1a shows that kitten No. 1 contains all the RD-114-related information, but only half the number of copies (C₀t_{1/2} 280), like the F₁ parent, whereas kitten No. 2 (from the same litter) lacks RD-114-related DNA sequences like its leopard cat parent. These results suggest that the multiple copies of RD-114 re-

Table 1 Segregation of RD-114 and FeLV type C viral sequences in various cats

Cats*		RD-114		FeLV	
		C ₀ t _{1/2} †	Average no. viral copies‡	C ₀ t _{1/2}	Average no. of viral copies
Domestic cat	No. 1	130	12–13	230	8–9
	No. 2	120	12–13	260	7–8
	No. 3	170	10–11	280	7–8
CCC clone 6§		150	10–11	250	7–8
European wildcat		140	11–12	210	8–9
F ₁ hybrid	No. 1	350	5–6	550	3–4
	No. 2	330	5–6	400	4–5
	No. 3	275	6–7	500	3–4
	No. 4	300	6–7	450	3–4
Backcross	No. 1	280	6–7	520	3–4
	No. 2	—	0	—	0
Leopard cat	No. 1	—	0	—	0
	No. 2	—	0	—	0
Cell lines					
RD-114/FCf2Th clone 10§		1,800	1	—	0
FeLV/FCf2Th§		—	—	1,900	1

* Cellular DNA was extracted from various organs (spleen, liver, kidney, lung) and hybridised to RD-114 and FeLV ³H-DNA as described in Fig. 1. Domestic cats Nos 2 and 3 were from the germ-free colony of cats at Merck, Sharp and Dohme; animals from this colony have never been found to be positive for infectious feline leukaemia virus. F₁ hybrid cats Nos 1, 2 and 3 and 4 belong to three separate litters.

† C₀t_{1/2} values represent the midpoint of the reannealing curves¹⁷.

‡ The approximate number of copies per haploid genome of sequences related to either RD-114 or FeLV were estimated from reciprocal plots (Fig. 1). The number of copies is determined by the ratio of the slope of each line to the slope of the line described by the reassociation of non-repetitive domestic cat cellular DNA (C₀t_{1/2} = 1,800–2,000; see also ref. 19). In the case of RD-114, where two sets of viral sequences can be detected in cellular DNA, the number of copies listed is the average of the two populations.

§ CCC clone 6 is from a continuous line of domestic cat kidney fibroblasts and is not releasing type C virus^{2, 3}, and RD-114/FCf2Th and FeLV/FCf2Th are a dog thymus cell line infected, respectively, with RD-114 (ref. 1) or with the helper virus from the Gardner–Arnsstein²³ strain of feline sarcoma virus.

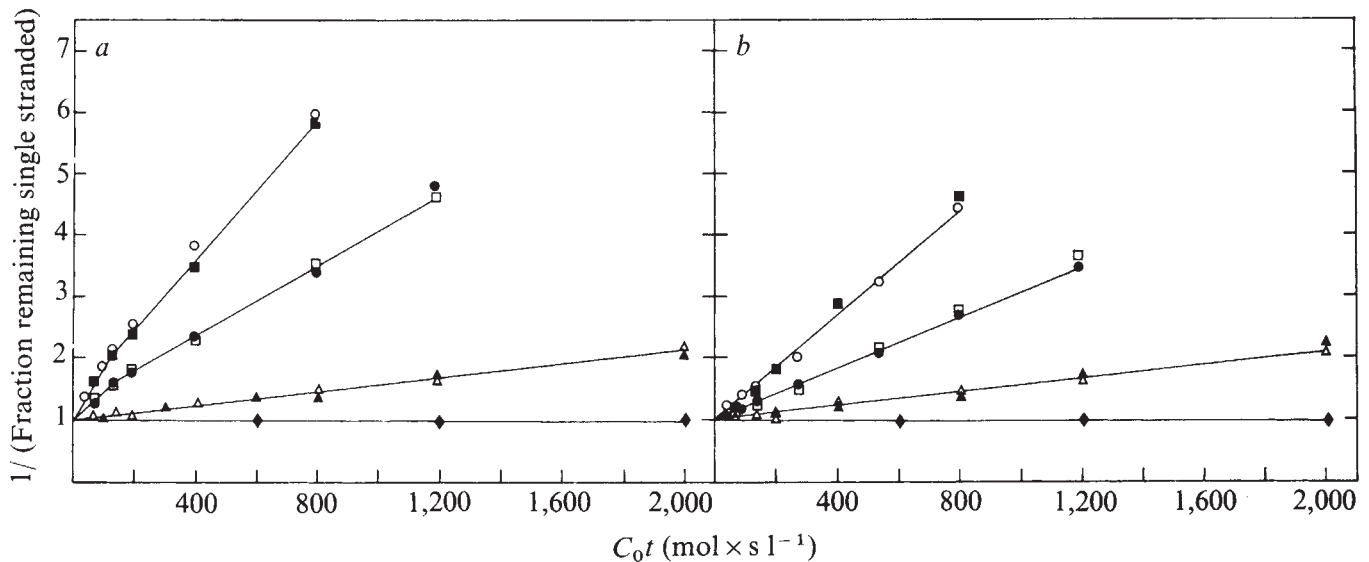


Fig. 1 Analysis of reassociation kinetics by the method of Wetmur and Davidson¹⁸ of RD-114 and FeLV ³H-DNA probes to cat cellular DNAs, and of domestic cat unique sequence cell DNA self-association. The ³H-thymidine-labelled DNA probes were synthesised from detergent-disrupted type C virus in the presence of actinomycin D as described²⁷. The specific activity of the ³H-DNA was 1.7×10^7 c.p.m. μg^{-1} . The ³H-DNA probes contained 60–66% of their respective 70S viral RNA sequences at a ³H-DNA:³²P viral RNA molar ratio¹⁰ of 2.0. Cellular DNA was extracted from tissues and cell lines as described¹⁰. All DNAs were treated sonically so as to yield a mean size of 6–8S (the size of the ³H-DNA probes)¹⁰. DNA:DNA hybridisations were incubated at 65 °C in reaction mixtures containing 0.01 M Tris, pH 7.4, 0.75 M NaCl, 2×10^{-3} M EDTA, 0.05% sodium dodecyl sulphate, 30,000 to 40,000 c.p.m. of ³H-DNA and 2–4 mg of cellular DNA per ml. Hybridisations were started by heating the mixtures to 100 °C for 10 min, cooling on ice to 4 °C and incubating at 65 °C. At various times, 0.025 ml portions were removed and frozen at –80 °C until digested with the single-strand specific nuclease, S₁, as described²⁷. C_0t values (C_0 is the concentration of cellular DNA in mol l^{-1} and t is the time in s) were calculated as suggested by Britten and Kohne²⁸ as $(A_{260} \text{ ml}^{-1})/2 \times h$, and corrected to a monovalent cation concentration of 0.18 M (ref. 29). **a**, Annealing of RD-114 ³H-DNA probe to DNA extracted from: ○, domestic cat No. 3; ■, domestic cat No. 1; ●, F₁ hybrid No. 4; □, backcrossed kitten No. 1; ▲, a dog thymus cell line infected with RD-114, and ◆, leopard cat and backcrossed kitten No. 2. Comparable data were obtained with DNA probes prepared from the domestic cat type C virus, CCC^{2,3}, and with the FS-1 virus isolated from European wildcat cells³⁰. △, Self-association of non-repetitive domestic cat cellular DNA. ³H-thymidine-labelled domestic cat cellular DNA was isolated by removing the highly reiterated sequences that anneal by a C_0t of 800 (approximately 40% of the total DNA) by fractionation on hydroxyapatite¹⁰. This ³H-thymidine-labelled DNA (5×10^9 c.p.m. μg^{-1}) was then hybridised to total domestic cat cellular DNA. **b**, Annealing of FeLV ³H-DNA probes (Rickard strain)²² to DNA extracted from: ○, domestic cat No. 2; ■, domestic cat No. 1; ●, F₁ hybrid No. 3; □, backcrossed kitten No. 1; ▲, a dog thymus cell line infected with FeLV, and ◆, leopard cat and backcrossed kitten No. 2. Comparable data were obtained with the Gardner–Arnstien²³ strain of FeLV grown in a canine thymus cell line (FCf2Th). △, Self-association of unique sequence domestic cat cellular DNA. Domestic cat cellular DNA contains at least two distinct sets of RD-114-related sequences which are present in different reiteration frequencies. The F₁ hybrid and backcrossed kitten No. 1 have approximately half the number of copies of each set of related sequences. While only one set of virogene sequences can be detected with the FeLV probe (Fig. 1b), the F₁ hybrid and backcrossed kitten No. 1 again are shown to contain half the number of copies as that found in the domestic cat parent.

lated sequences are located together at one (or relatively few) chromosomal sites.

The same cats were examined for FeLV-related genes using ³H-DNA probes prepared from various strains of FeLV^{22,23}. No cross-hybridisation between these probes and RD-114 is detectable^{15,24}. The results parallel exactly the data obtained with transcripts of RD-114 RNA. The domestic cat parent contains multiple copies of FeLV-related virogenes whereas the leopard cat parent lacks these sequences. The F₁ hybrids contain half the number of copies. Backcrossed kitten No. 1 has the same number of virogene copies as its parent (the F₁ hybrid) although its littermate has no detectable sequences related to FeLV.

Table 1 summarises the hybridisation data; four clear classes of DNAs are evident. The first consists of animals that contain full complements of RD-114 and FeLV-related gene sequences. These cats contain both sets of virogene sequences reiterated a comparable number of times, although there may be fewer FeLV-related copies. The animals in the second class, including all the F₁ hybrids and one of the backcrossed kittens, contain half the virogene complement of the RD⁺, FL⁺ parents. A third class contains sequences that anneal to the viral probe with reassociation kinetics comparable with that seen for the association of the most slowly reannealing cellular DNA sequences and therefore probably contains one viral copy. This includes cloned heterologous cell lines producing high titres of either RD-114 or FeLV viruses. The fourth class completely lacks sequences related to either RD-114 or FeLV and includes the leopard cats and one of the backcrossed kittens.

If the multiple copies of RD-114 and FeLV-related sequences present in cat DNA had become physically separated from one another after their entry into the cat germ line⁹ they would not all have segregated together. The data rule out the possibility

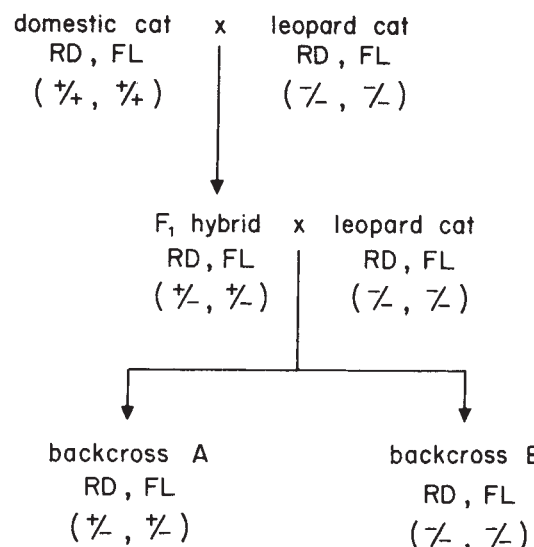


Fig. 2 RD-114 and FeLV type C viral genotypes of the various cats studied.

that each of the multiple copies of RD-114 or FeLV-related virogenes occurs on a different linkage group, as well as models of non-chromosomal inheritance of the multiple viral copies.

Since only certain *Felis* species contain RD-114 and FeLV-related sequences in their DNA, we propose that both classes of viruses were acquired by cats subsequent to their major radiation, most likely in the Pliocene, and that both sets of sequences have been perpetuated in the germ line^{9,16}. The multiple viro gene copies presumably arose as a result of gene duplication and/or unequal crossing-over after infection. The presence of multiple copies of both sets of virogenes in cat cellular DNA seems to be a general property of endogenous mammalian type C viruses; mouse, rat, hamster, pig and baboon DNAs also contain similar numbers of copies of their respective endogenous viruses¹⁹.

The genetic crosses described here provide a new approach to the study of the evolution of multiple gene systems. The viro gene sequences can be considered as one of the group of moderately repetitive sequences such as the genes for 5S RNA²⁵, histones²⁸ and feather keratin²⁰. The existence of natural populations of animals that either lack or contain DNA sequences related to both RD-114 and FeLV, and the ability of these cats to interbreed permits the study of the physiological and potentially pathological role of each of these genetically transmitted gene sequences. Hybrid animals containing half the number of viro gene copies and viro gene-negative cats should allow the study of the effects of gene dose on susceptibility and resistance to diseases mediated by both groups of type C viruses.

We thank J. Koci and G. L. Wilson for assistance, and R. Callahan and C. Sherr for comments and discussion. Supported in part by the Virus Cancer Program of the National Institutes of Health.

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Received June 30; accepted August 26, 1975.

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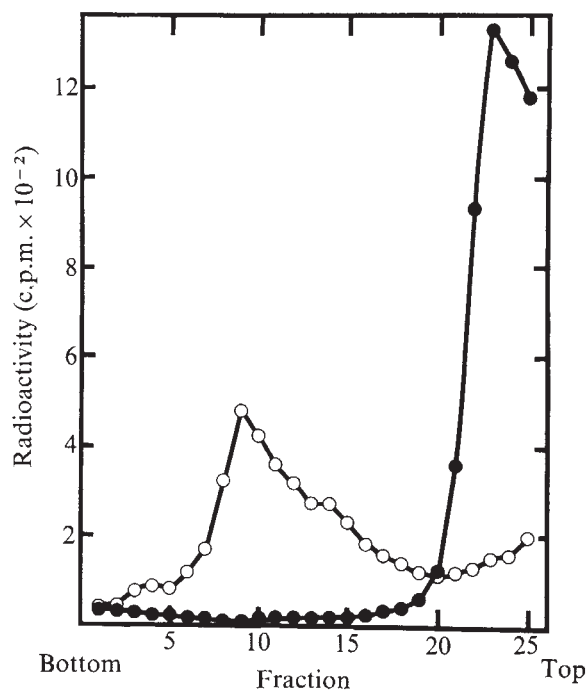
Identification of heat-dissociable RNA complexes in two porcine coronaviruses

THE coronavirus genome has been shown to comprise single-stranded RNA^{1,2}. Examination of the viral nucleic acid synthesised by pig kidney cells infected with transmissible gastroenteritis virus (TGEV) suggested that several molecular species, ranging in size between 18 and 28S, were involved in the viral replicative cycle³; similarly Tannock found a wide variation in the size of RNA molecules extracted from avian infectious bronchitis virus (IBV) by a phenol-sodium dodecyl sulphate (SDS) method². Extraction of IBV RNA by 1% SDS at 60 °C has, however, revealed a single component of molecular weight 9×10^6 corresponding to 60S by electrophoresis through 2.2% polyacrylamide gels⁴.

We have examined the RNA extracted from purified preparations of TGEV and a second porcine coronavirus—haemagglutinating encephalomyelitis virus (HEV)—and have found a 60-70S RNA component which dissociates into 35S and 4S material on heating above 60 °C in a way that closely resembles the genome of the oncogenic RNA viruses.

We had observed that treatment of purified TGEV with 1% SDS at 20 °C disrupted the virions and liberated a high molecular weight complex containing the RNA. On the assumption that this complex might comprise the hitherto undetected ribonucleoprotein, we extracted the material from TGEV preparations radioactively labelled with ³H-uridine or with ³H-leucine to determine which structural polypeptide was associated with the complex. As is shown in Fig. 1, however, the fast moving RNA complex has no detectable protein associated with it, while polyacrylamide gel analysis of the radioactivity remaining near the top of

Fig. 1 Rate zonal sedimentation of TGEV after treatment with 1% SDS at 20 °C. TGEV was grown in secondary pig thyroid cell (APT/2) cultures in the presence of 5-³H-uridine (○) or 4,5-³H-leucine (●) and purified by sucrose gradient centrifugation as described previously⁵. After treatment with 1% SDS at 20 °C for 15 min, each preparation was layered over a 6-ml 15-30% (w/w) sucrose gradient and centrifuged at 250,000g for 2 h in a swing-out rotor. The gradients were fractionated by siphon and total radioactivity was determined for each fraction.



the gradient demonstrated the presence of viral structural polypeptides⁵.

Extraction of RNA from ³H-uridine-labelled TGEV by 1% SDS at 20 °C followed by electrophoresis through gels containing 2% polyacrylamide and 0.6% agarose revealed a homogeneous band of high molecular weight RNA and a very small amount of radioactivity in the area of the marker dye, corresponding to approximately 4–7S (Fig. 2a). Similar extractions were performed on replicate virus samples using 1% SDS at 40, 60, 80 and 100 °C and the released RNA was electrophoresed as before. The electrophoretograms illustrated in Fig. b–e showed that the mobility of the major RNA band increased slightly as the extraction temperature was raised to 60 °C and, while the homogeneity of the leading edge of the band decreased somewhat, there was no obvious change in the 4S–7S region. As the temperature was increased through 80 to 100 °C, however, the radioactivity became associated with a broader band of RNA, whose mobility was at least twice that of the RNA extracted at lower temperatures, and there was an increase in the amount of RNA in the 4S region of the gel.

This apparent "melting" of the high molecular weight complex into smaller components with the liberation of 4S

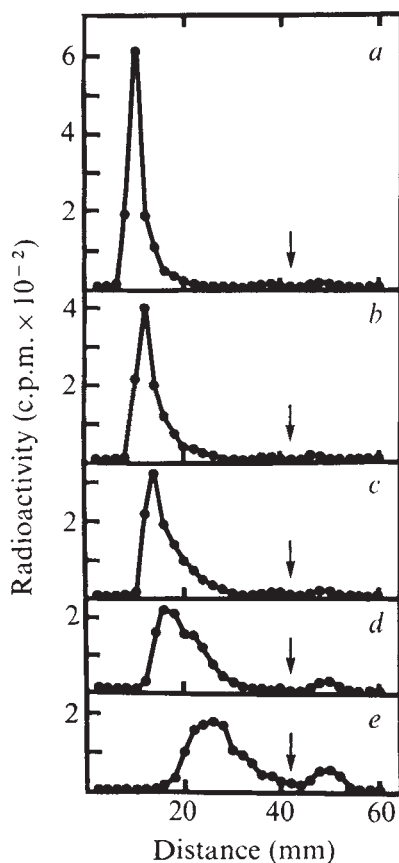


Fig. 2 Gel electrophoresis of TGEV RNA following extraction in 1% SDS at various temperatures. TGEV was grown in APT/2 cultures in the presence of ³H-uridine and purified by sucrose gradient centrifugation. The preparation was suspended in L buffer (36 mM 2-amino-2-hydroxymethylpropane-1,3-diol (Tris); 30 mM NaH₂PO₄; 1 mM ethylenediaminetetraacetic acid (EDTA); pH 7.8) and SDS was added to 1% at 20 °C. The suspension was divided into five parts and each part was held in a water bath at 20 (a), 40 (b), 60 (c), 80 (d) or 100 °C (e) for 30 min. After cooling, sucrose was added to 5% (w/w) and bromophenol blue was added as a marker dye, each sample was layered over a 6 × 60 mm 2% (w/v) polyacrylamide/0.6% agarose gel⁶ and electrophoresed for 1 h at 8 mA per gel using L buffer containing 0.2% SDS. The gels were sliced into 1-mm fractions and the radioactivity of each slice was determined in a toluene-Triton X-100 scintillant. The location of the marker dye was estimated by eye and is represented by the arrows.

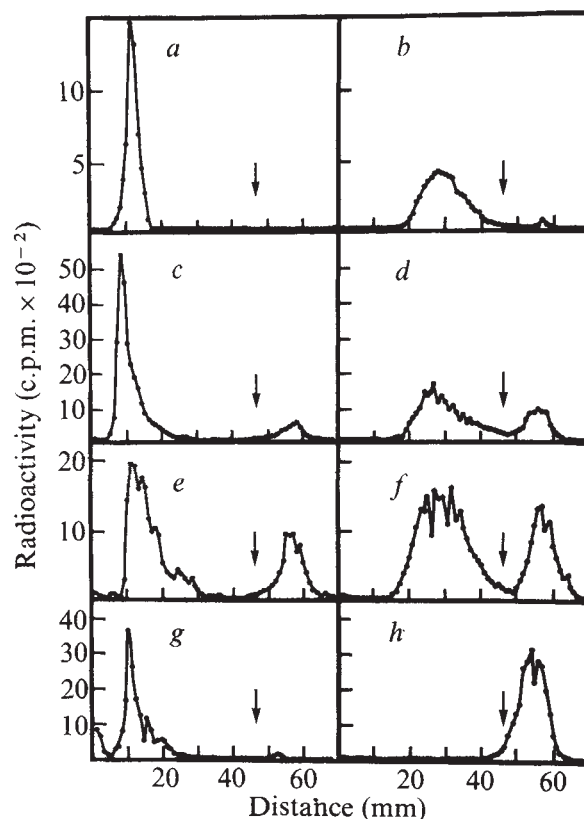


Fig. 3 Gel electrophoresis of RNA from RSV, TGEV, and HEV. TGEV and HEV were grown in APT/2 and primary pig kidney cell cultures respectively in medium containing 5-³H-uridine. Both viruses were purified by sucrose gradient centrifugation and suspended in L buffer. After 24 h at 4 °C or, in the case of the virus used in (g) and (h), after 20 d at 4 °C, SDS was added to 1% and the preparations were held at 20 or 100 °C for 30 min. Purified 60–70S RNA from RSV was held in 10 mM NaCl; 8 mM Tris; 7 mM NaH₂PO₄; 1 mM EDTA; 1% SDS; pH 7.8 at 20 and 100 °C for the same length of time. The preparations were cooled to 20 °C, sucrose and bromophenol blue were added and electrophoresis and fractionation were conducted as described for Fig. 2, using 6 × 70 mm gels. RSV RNA held at 20 (a), and 100 °C (b); TGEV extracted at 20 (c), and 100 °C (d) after 24 h at 4 °C; HEV extracted at 20 (e), and 100 °C (f) after 24 h at 4 °C; TGEV extracted at 20 (g) and 100 °C (h) after 20 d at 4 °C. The arrows denote the location of the marker dye.

RNA at temperatures above 60 °C closely resembles the findings for the oncogenic RNA viruses⁷. To determine the size of the coronaviral RNA components and their similarity to oncornaviral RNA, we compared the mobilities of RNA extracted from TGEV and HEV at 20 and 100 °C with those of purified Rous sarcoma virus (RSV) RNA held at the same temperatures. Figure 3a–f shows that, by this method, the RNA complexes extracted from the two coronaviruses are indistinguishable in size from the 60–70S component of RSV RNA and that, after heating, the TGEV and HEV RNA components are comparable in size to the RSV 35S RNA. There seems to be more heterogeneity in the coronaviral 35S RNA band than in the RSV equivalent and this holds true regardless of the time of the labelling period. Comparison of RNA extracted from TGEV labelled with ³H-uridine 0–4, 4–8, 8–12, 12–16 or 0–20 h after infection shows that, although the total radioactivity incorporated varied with the time of labelling period, the overall shape of the RNA curves after extraction at 20 and 100 °C were similar. This difference in heterogeneity may be due partly to the fact that we were comparing extracts from whole HEV and TGEV with purified 60–70S RNA from RSV, but it may also be caused by partial degradation of the RNA within the virion. That this undoubtedly occurs is demonstrated by the electrophoretograms illustrated in Fig. 3g–h, which were all derived

from TGEV that had been held at 4 °C for 20 d before extraction at 20 and 100 °C in 1% SDS. The 60–70S complex seems to be intact, but, on melting, the complex liberates only small fragments of RNA of approximately 4S (Fig. 3h). This suggests that the virus preparations have an associated ribonuclease capable of producing breaks in the 35S strand while they are complexed in the 60–70S form. Whether the large amount of 4S RNA detected in all HEV preparations so far examined (Fig. 3e–f) represents degraded viral RNA or host tRNA associated with the virions is not known.

Our inability to detect protein in the 60–70S RNA complex from TGEV does not exclude the possibility that there is a very small amount that is dissociating from the RNA at elevated temperatures in the presence of SDS. The similarity of behaviour and size between the coronavirus RNA and RSV RNA, together with the liberation of 4S RNA on melting suggests strongly that the TGEV and HEV 60–70S complex is held together by RNA–RNA interactions as is the RNA from oncornaviruses. We hope to characterise further the 60–70S complex and determine whether the viral 4S component is in fact host tRNA. Although the replication of these two groups of viruses is fundamentally different, the coronaviruses being entirely cytoplasmic in contrast to the essential nuclear phase of the oncornaviruses, a similarity in the structure of the genomes of the two groups raises interesting implications for the phylogeny of the RNA tumour viruses.

We thank Mrs Brenda V. Pike for assistance and Dr S. Martin, Imperial Cancer Research Fund Laboratories, London, for the gift of ³H-uridine-labelled RSV RNA. One of us (T.M.W.) was in receipt of a British Council Scholarship.

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Received June 20; accepted August 20, 1975.

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Presence of factor VIII-related antigen in blood platelets of patients with Von Willebrand's disease

VON WILLEBRAND'S disease (VWD) is an autosomally inherited disorder characterised by low factor VIII activity (antihaemophilic factor, AHF), prolonged bleeding time, reduced retention of platelets in a glass bead column and abnormal distocetin-induced platelet aggregation. The prolonged bleeding time in VWD has been attributed to the absence of a plasma factor, the von Willebrand factor (VWF), as shown by a correction of the bleeding time after infusion of normal and haemophilic plasmas¹. Addition of purified factor VIII *in vitro* specifically corrects the abnormal platelet retention and ristocetin aggregation in VWD^{2–4}, whereas transfusion of similar material into dogs with VWD also corrects the prolonged bleeding time (B.N.B., W. J. Dodds; J. A. van Mourik, J.J.S. and

W. P. Webster, unpublished). This led to the suggestion that factor VIII is closely related if not identical to VWF, although dissociation of factor VIII procoagulant activity from factor VIII-related antigen (F VIII-RA) is observed in certain conditions^{6,7}. The plasma concentration of F VIII-RA is usually reduced in VWD suggesting a reduced synthesis of factor VIII (VWF)⁸. In contrast to this we now report the presence of normal concentrations of F VIII-RA in platelets of patients with VWD. This F VIII-RA supported aggregation induced by ristocetin in a washed platelet system⁹, a property of factor VIII which has been attributed to VWF activity.

Human blood platelets were washed according to the method of Karparkin¹⁰. ACD-blood was centrifuged (10 min, 200g, 20 °C). Platelet rich plasma was diluted in 5 volumes of Krebs-Ringer buffer (pH 7.4) containing 9 mM Na₂-EDTA. After centrifugation (15 min, 1,000g, 4 °C), the platelets were washed once in Krebs-Ringer buffer containing 9 mM Na₂-EDTA and twice in Krebs-Ringer buffer containing 1 mM Na₂-EDTA. The platelet pellet was finally resuspended in Krebs-Ringer buffer containing 30 mM glucose and 1 mM ε-amino caproic acid. In the final washing fluid the protein content was lower than 50 μg ml⁻¹ (ref. 11), and the concentration of F VIII-RA, measured by electroimmunodiffusion was below 0.05 U ml⁻¹ (ref. 12). 1 U F VIII-RA was defined as the amount present in 1 ml pooled normal plasma prepared from 40 healthy subjects¹². In the final suspension (1×10^6 – 3×10^6 platelets μl⁻¹) the platelets were disrupted by freezing and thawing (four times) followed by centrifugation (60 min, 30,000g, 4 °C).

Ten suspensions of normal human platelets were tested. The concentration of F VIII-RA detected in the supernatant was 0.15 U mg⁻¹ platelet protein (range 0.11–0.25) or 43 U per 10¹¹ platelets (range 19–94). Expressed per platelet volume (8×10^{-15} l) (ref. 13) the concentration of F VIII-RA was 60 times (range 21–156) higher than that in plasma. Similar values were reported by Nachman and Jaffe¹⁴.

Platelet F VIII-RA showed a reaction of identity with plasma F VIII-RA, when tested in immunodiffusion using a rabbit anti-factor VIII serum and the antisera raised against the low ionic strength components (ref. 15 and B.N.B., J. van Mourik, S. de G., J.M.H.-H. and J.J.S., unpublished). Cross-immunoelectrophoretic analysis revealed an electrophoretic mobility comparable with that of plasma F VIII-RA.

Howard *et al.*¹⁶ quantitated F VIII-RA on intact washed normal platelets and concluded that F VIII-RA was firmly bound to the membrane fraction. A similar conclusion was reached by Bloom¹⁷ using an immunofluorescent technique. On the other hand, the localisation of F VIII-RA throughout the megakaryocytic cytoplasm is in agreement with its localisation inside the platelet¹⁸. Nachman and Jaffe¹⁴ reported the presence of F VIII-RA in subcellular membrane and granula fractions. We examined this localisation by a previously described indirect immunofluorescent technique^{19,20}. Suspensions of intact washed platelets were incubated with antifactor VIII in suspension, washed again, and incubated with fluorescein isothiocyanate labelled horse anti-rabbit globulin (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam). A drop of this suspension was studied under the fluorescence microscope. Most platelets were unstained, whereas a vivid granular staining was obtained after disruption of membranes by air drying of a drop of the same platelet suspension on a glass slide. The specificity tests have been described in detail elsewhere²⁰. Staining with normal rabbit serum instead of with antifactor VIII was used as a control. The difference between staining in suspension or staining after air drying suggests that F VIII-RA is present inside the platelets.

In agreement with the results of Howard¹⁸ and Nachman and Jaffe¹⁴ no factor VIII activity could be detected in

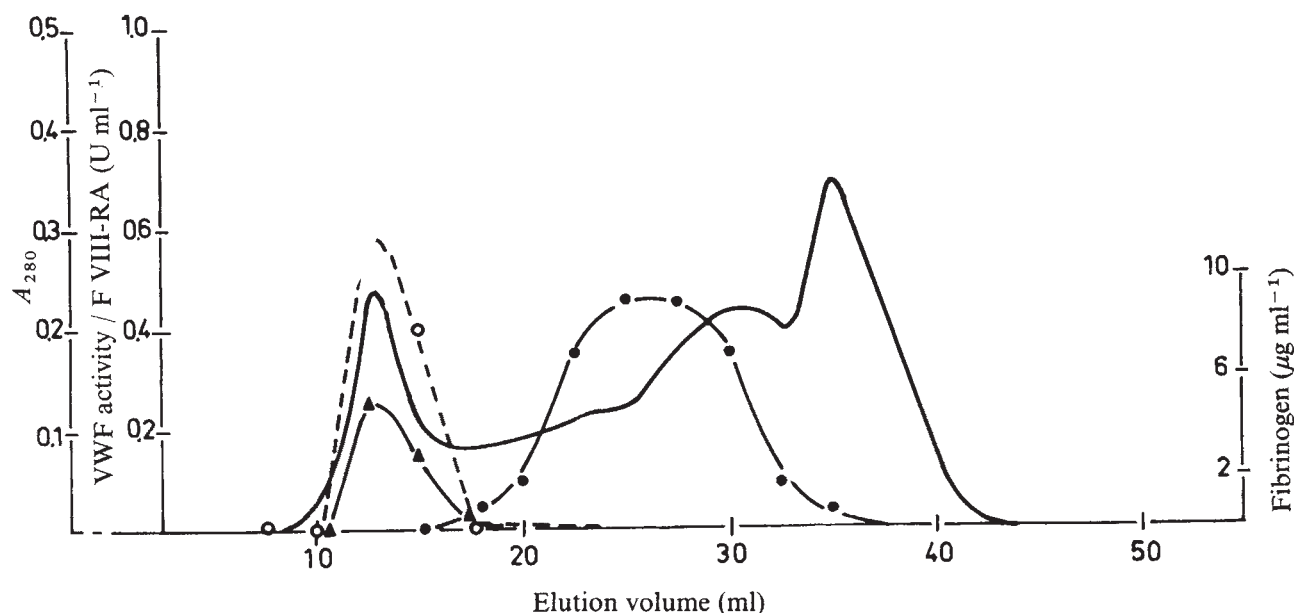


Fig. 1 Chromatography of a platelet lysate obtained from a patient with VWD. A column (K15/30, Pharmacia, Uppsala) was packed to a gel height of 24 cm with Sepharose 6B (Pharmacia, Uppsala) and equilibrated with a barbitol-saline buffer (0.0167 M barbitol, 0.125 M NaCl, pH 7.0) containing 3.3% dextran (Rheomacrodex, molecular weight 43,900, Pharmacia AB) and 1 mM ϵ -amino caproic acid. Platelet lysate obtained from 70 ml of normal human blood or blood from patients with VWD in a final volume of 1–2 ml was applied to the top of the column and the column was eluted with buffer at 15 ml h^{-1} . All procedures were performed at 4 °C. Fractions were analysed for: (1) absorbance at 280 nm (—); (2) F VIII-RA as measured by Laurell technique ($\circ$)¹²; (3) fibrinogen as measured by Laurell technique ($\bullet$)²⁷; and (4) presence of VWF activity as determined by support of ristocetin aggregation in washed platelet suspensions ($\blacktriangle$ — $\blacktriangle$)⁹.

the supernatant of platelet lysates. It should be noted, however, that a clot-promoting activity was detected using a one stage factor VIII assay²¹. This clot-promoting activity could not be inhibited by human circulating factor VIII inhibitors, and was also detected in the supernatant of lysed haemophilic platelets. The clot promoting activity was therefore not attributed to factor VIII and probably resulted from tissue factor activity released by contaminating leukocytes. The lack of factor VIII activity could not be attributed to the freezing and thawing procedure, because factor VIII activity was already absent after the first freeze-thaw manipulation. The presence of proteolytic enzymes, as a cause of the decreased factor VIII activity is more difficult to exclude. However, addition of 1 mM ϵ -amino caproic acid or other proteolytic inhibitors (benzamidine, 1 mM; Soya bean trypsin inhibitor, 0.02 mg ml^{-1} ; and phenyl methyl sulphonyl fluoride, 1 mM) to the final washing fluid before disruption of the platelets had no effect on the measurement of factor VIII activity. Moreover the supernatant obtained after freezing and thawing did not inactivate the clot promoting activity of purified factor VIII during 24 h of incubation at 37 °C. This indicates that the factor VIII identified in platelets as F VIII-RA is biologically inactive. Similar observations were made for human endothelial cells, FVIII-RA was present¹⁸ but devoid of factor VIII activity²².

The presence of F VIII-RA has been demonstrated within haemostatic plugs²⁰. Electron microscopic observations of haemostatic plugs in rabbits and dogs have shown that the platelets are mostly degranulated²³. In preliminary investigations we found that collagen as well as thrombin (final concentration 5 U ml^{-1} , Roche, Basle) produced little or no release of F VIII-RA from intact platelets which explains the presence of F VIII-RA in haemostatic plugs that had undergone release.

Normal concentrations of F VIII-RA were detected in platelets from patients with haemophilia A. F VIII-RA was also measured in platelets from 15 patients with VWD (Table 1). All patients except No. 10, who was considered to manifest VWD type II (ref. 24), had low or absent

F VIII-RA in their plasma and fulfilled the criteria of VWD. In two patients (Nos 4 and 11) no F VIII-RA was detected in the platelets, whereas all other patients had normal concentrations of F VIII-RA in their platelets. These results were supported with an indirect immunofluorescence technique¹⁹ applied to drops of platelet suspensions air-dried on a glass slide. A positive result was obtained with all patients, except one (No. 4) of the two patients without F VIII-RA in their platelets. The other patient was not tested. It is of interest that the patient (No. 11) without F VIII-RA in his platelets is a brother of patient No. 15, suggesting that the absence of F VIII-RA in platelets of patients with VWD is not hereditary.

F VIII-RA from normal human platelets was purified by gel chromatography according to van Mourik and Mochtar²⁸ (Fig. 1). Both F VIII-RA and VWF-activity were detected in the void volume (V_0) fractions from these columns, which is in agreement with observations for plasma factor VIII³. Figure 1 shows the results for the chromatogram lysates of VWD platelets. In 3 experiments, the V_0 fractions contained both F VIII-RA and VWF-activity, whereas no VWF-activity and F VIII-RA was detected in the V_0 of VWD platelet lysates containing no F VIII-RA.

These results indicate that although F VIII-RA is reduced or absent in the plasma of VWD patients, F VIII-RA can be detected in the platelets of most of these patients.

Two reports in the literature are in conflict with our results. Howard *et al.*¹⁶ failed to demonstrate any F VIII-RA in the platelets of one patient with severe VWD and Coller *et al.*²⁸ found platelets from two patients to be negative in immunofluorescence studies. Shearn *et al.*²⁹, however, comparing five different heterologous antifactor VIII antisera found that the platelets from one patient with severe VWD were positive with four of the five antisera using an immunofluorescence technique. The presence of the antigen was also demonstrated by cross immunoelectrophoresis. It should be noted that these studies were performed with a very limited number of patients, whereas the techniques used also differ from ours. One explanation for the discrepant results, may be contamination of the antifactor VIII sera

Table 1 Platelet factor VIII-related antigen (F VIII-RA) in VWD

Patient	FVIII-RA/mg platelet protein (U)	FVIII-RA/10 ¹¹ platelets (U)	FVIII activity plasma (U ml ⁻¹)	FVIII-RA plasma (U ml ⁻¹)	Bleeding time ²⁵ (min)
1	0.18	35	0.14	< 0.05	7
2	0.14	56	0.23	< 0.05	9
3	0.04	16	0.13	< 0.05	7
4	< 0.01	< 0.01	0.12	< 0.05	> 15
5	0.15	35	0.11	< 0.05	—
6	0.27	60	0.05	< 0.05	> 15
7	0.18	52	0.15	< 0.05	> 15
8	0.26	67	0.12	< 0.05	10
9	0.23	90	0.18	< 0.05	—
10	0.24	47	0.44	0.80	> 15
11	< 0.01	< 0.01	0.15	0.10	—
12	0.08	14	0.10	0.10	—
13	0.14	38	0.11	0.05	—
14	0.36	120	0.35	0.40	9
15	0.16	34	0.21	0.25	6

with antibodies produced in rabbits against platelet fragments, which may be carried along in the purification of factor VIII. The following observations suggest that in our experiments the antigen detected by rabbit anti-factor VIII in platelets is indeed F VIII-RA.

(1) The platelet antigen could not be distinguished from plasma factor VIII when tested in immunodiffusion and cross immunoelectrophoresis, using antifactor VIII and the antisera against the low ionic strength components of factor VIII. (2) The positive immunofluorescence of haemostatic plugs was completely blocked by absorption of antifactor VIII with purified factor VIII, the characteristics of which have been described in detail^{15,26}. (3) In preliminary experiments antibodies were prepared against normal and VWD platelet F VIII-RA. These antisera precipitated with purified factor VIII when tested in immunodiffusion experiments.

Previously it has been suggested that the capacity to synthesise F VIII-RA is reduced in VWD. This was concluded from the fact that F VIII-RA is reduced or absent in plasma of patients with VWD. Our findings, however, suggest that the platelets, or more likely the megakaryocytes retain the capacity to synthesise F VIII-RA. Another possible explanation for the presence of F VIII-RA in VWD platelets is that the platelets selectively sequester F VIII-RA from an extracellular source such as plasma or endothelial cells. The fact that F VIII-RA from normal as well as from VWD platelets supports ristocetin-induced aggregation may indicate that platelet F VIII-RA is also identical to VWF. VWF has been deemed necessary for normal haemostasis because patients with VWD who lack this factor have a prolonged bleeding time. The presence of F VIII-RA in haemostatic plugs²⁰, and blood platelets suggests that it serves some useful function in haemostasis. A direct haemostatic role of platelet F VIII-RA seems unlikely because it is not released from intact platelets and is present in platelets of patients with VWD who have prolonged bleeding times. Although plasma F VIII-RA seems to be more important in haemostatic plug formation the presence and synthesis of F VIII-RA in endothelial cells and its localisation in blood platelets suggest additional haemostatic functions for this molecule.

We thank Dr Jean Dodds for criticising this manuscript. This investigation was supported in part by the Foundation for Medical Research (Fungo) which is subsidised by the Netherlands Organization for the Advancement of Pure Research (ZWO).

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Corrigendum

In the article "The origin of nuclei and of eukaryotic cells" by T. Cavalier-Smith (*Nature*, **256**, 463; 1975) the two unlabelled arrows at the top right of Fig. 5 should read (from the top) Prasinophyta and Charophyta, respectively.

Erratum

In the article by B. Donzel and M. Goodman (*Nature*, **256**, 750; 1975) the title should have read "Synthesis and conformations of hypothalamic hormone releasing factors: two TRF analogues containing backbone N-methyl groups" and not as printed.

matters arising

Strainmeter technology

WE agree with Sydenham¹ that many problems remain to be investigated with strainmeters and that many results obtained so far are ambiguous or contradictory. Measurements of strain (especially long period strain) are beset by a host of problems not found in such areas as magnetometry, so it may, therefore, be unreasonable to expect strainmeters to be as simple as magnetometers. Further, it may be time to opt for a smaller number of higher performance instruments and to reconsider the continued deployment of more and more strainmeters in a given area.

Although the measurement of strain is in principle differential, in practice the long term nature of most strain measurements requires great stability in the reference length, the realisation of which is tantamount to constructing a length standard.

Conventional materials, such as fused silica, have coefficients of expansion of about $10^{-6} \text{ }^{\circ}\text{C}^{-1}$ so that thermal fluctuations must be held to less than $10^{-4} \text{ }^{\circ}\text{C}$ over the entire length of the instrument if the thermally induced strain signal is to be kept below 10^{-10} (in order to measure a particular component of the Earth tides to an accuracy of 1%, for example).

Sydenham has shown³ that this can be done for very short strainmeters (10 m) in the laboratory. The ancillary apparatus required to achieve microdegree stabilities is, however, far from simple, even for 10-m instruments, and difficulties in stabilising longer instruments can be expected to increase more than linearly with length.

The length of fused silica strainmeters also depends on the ambient humidity in a nonlinear manner (F. Homuth, unpublished thesis). Thus, stabilisation of the humidity is also required. That could be accomplished, at least to the first order, by enclosing the instrument in an evacuated pipe, in which case, of course, it would look exactly like a laser strainmeter.

By way of contrast, saturated absorption stabilisers routinely achieve stabilities and reproducibilities of 10^{-11} or better without any sort of thermal or environmental isolation. These data on stability and reproducibility are obtained by direct comparison between two independently stabilised devices.

It is true that the interferometer comprising the heart of the laser strainmeter is sensitive to fluctuations in atmospheric pressure and temperature, the fractional

change in the optical path length being 2×10^{-10} per mtorr. A simple mechanical pump can easily evacuate the interferometer to a few mtorr and can maintain the pressure at that level indefinitely. The temperature dependence of the index of refraction at a few mtorr is negligible.

Until the advent of saturated absorption stabilisers, attempts to measure long term strain accumulations with conventional strainmeters were severely limited by the signals produced by various environmental effects. There is no question that conventional strainmeters can be used for conventional seismology and even for normal mode work (periods of less than about 1 h). In fact, many of the best normal mode data come from quartz rod strainmeters⁴. But for periods of longer than 1 h it becomes more and more difficult to isolate adequately the reference length.

Thus, our conclusions are quite different from those of Sydenham. We feel that there is no 'best' overall strainmeter, and that each instrument has advantages and shortcomings.

Laser strainmeters are usually faulted for their high cost and low reliability. But neither the cost nor the sophistication of a laser instrument increases very much with length, so that long instruments are quite a bit cheaper per metre than are short ones. We suggest that the opposite may be true for instruments requiring good thermal stability over their entire length. If, as Homuth (unpublished) suggests, stabilisation of the ambient humidity turns out also to be necessary, the cost of the total system will be even higher, and the reliability, presumably, lower.

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designed and constructed by Sydenham³ achieved signal-to-noise ratios of up to 100 on tidal peaks and although the instrument had defects, a poor signal-to-noise ratio was not the most important. As Sydenham has never published any power spectra it is not possible for others to compare the behaviour of their instruments with his. His interest in drift and stability amounts to an avid concern with only the lowest frequencies of the spectrum. Using drift as a criterion for comparison, however, it is interesting to note that Sydenham's early instrument has exhibited less long term drift than he now claims for quartz instruments⁴ although even that level is too high to permit a strainmeter to compare in the long term (more than 1 yr?) with geodetic techniques for determining secular strain.

Sydenham's discussion of tensor arrays is also misleading. Given six instruments and a good site, a tensor array will not usually give maximum useful information. Geophysically important results (different examples are given in refs 5–9) can be obtained using single instruments or linear arrays of horizontal strainmeters and these also reveal site effects^{7,10,11}. Should a site prove to be badly chosen perhaps it is best to go elsewhere.

No published data suggest that mechanical strainmeters are universally better than optical strainmeters (or vice versa). We register surprise that since Sydenham favours borehole instruments he makes no comment on the borehole dilatometer developed at the Carnegie Institution, Washington¹² or the Geotech borehole strainmeter¹³.

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SYDENHAM REPLIES—My paper was compiled in late 1973, and represents events of that time. A vital word—'headed'—did not appear at the end of the title as intended.

By early 1974, experimental Earth-strain research had reached a stage at which mechanical instrumentation was no longer a significant unknown of any programme. The next stage in maturity was to know how to obtain geophysical contributions with the hardware. In 1974, the modifying effects of cavities and regional topography were universally recognised and serious studies were initiated (earlier work with linear arrays was simplistic and often non-productive). Important and unexpected results have emerged from this approach (ref. 1 and D. P. Blair, unpublished thesis) showing how and to what extent expected strain magnitude is altered. Blair (unpublished), extending Harrison's⁴ finite-element work from two into three dimensions has found that topography selectively alters the amplitudes of the tidal spectral components. This result apparently explains that topography was the influencing parameter sought without success at the Queensbury site²; yet King questions my earlier predictions of the relevance of a tensor study.

Reference about strain-creep using linear arrays was indeed omitted as it is more usually studied using quite coarse instruments (though in my original manuscript it was suggested that creep theory, as studied by materials scientists, could be relevant to an understanding of creep mechanisms). This concept has since been invoked³.

I suggested that theory might help reduce the number of components needed to understand how strains relate at different places. Available techniques now allow the prediction of site effects using only a geological map, a topographical map and computing ability. We can now eliminate most known causes of incompatibility between theory and practice, leaving the interesting differences to be investigated. Deep-sea cotidal charts, for instance, might soon be updated using corrected Earth-strain data (D. P. Blair, unpublished thesis). Resolution of the interval argument could be near: structural studies based on topographical studies should give quantitative guidelines.

My work on drift reduction in instruments led to the development of the mechanical instruments King's group now uses. A very real advantage of high stability is the ease it offers of transducer design and recording. Quartz chain catenary instruments need no autoranging recorder nor any adjustments over yearly periods. Long-period differential drift

between two different quartz instruments is no greater than 5 parts in 10^{12} per hour⁴. Very long baseline interferometers excepted, no available geodetic technique can provide drift as low.

Frequency spectra could provide a means of assessing instrument performance but only if the power contributions of the Earth and the instrument can be distinguished separately. I have seen no point in publishing the tidal spectra for they contribute little. The work of Berger and Levine⁵ referred to by King represents excellent spectral analysis, but does it provide useful knowledge? If a site topography alters the amplitude¹ and composition of signal (ref. 1 and D. P. Blair, unpublished thesis) it does likewise to the tensor fluctuation noise. Comparisons could be out by a factor of 0–3 if site coefficients differ greatly.

Is not the ultimate strainmeter one that provides adequate quality data, ease of recording, ability to be used anywhere in any direction, is the cheapest, the quickest to install, creates the smallest cavity error, automatically compensates for any local thermoelastic effect, and gives high reliability? A core-hole instrument (D. Curtis and P. H. S., unpublished) offers that at the economic ratio of 150 to one (compared with the first kilometre-length surface laser instrument). Until laser instruments reveal significant geophysical information not gained by well designed, mechanical equivalent extensometers, I cannot see how it can be argued that they are worthwhile geophysical tools. Surely, Levine does not justify laser expenditure on cost per unit length at this stage of uncertainty about the ideal interval?

Levine's comments on environmental effects on quartz can be misleading. Ambient control in subsurface installations is usually just within needs. Even if quartz is unstable with humidity change, it is easy to control relative humidity with a passive flexible enclosure.

The cost of advanced total environmental control⁶ is no more than that of a supplying and running a fail-safe evacuated enclosure. I again stress the point, however, that this line of reasoning is not fundamentally correct. An instrument having the same thermal coefficient (made of stainless steel, not quartz) as the host rock in which it is buried, requires no ambient compensation.

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Extinction of taxa and Van Valen's law

VAN VALEN¹ has proposed an evolutionary law that has attracted considerable attention^{2–5}. He showed that plots of survivorship of taxa as a function of age are commonly log-linear (see Fig. 1 of ref. 1). This he interpreted to mean that the probability of extinction of a taxon is independent of its age, and thus formulated the law: "The effective environment of the members of any homogeneous group of organisms deteriorates at a stochastically constant rate."¹ We show here that survivorship analysis of the log-linear curves constructed by Van Valen does not warrant the interpretation that he assigned to it; support for Van Valen's law must be sought elsewhere.

In life table analysis one of two methods are used to construct a survivorship curve^{7–9}. Horizontal, dynamic, or cohort life tables require a set of optimisms of even age (usually taken at birth), whose survivorship curve is derived from a complete analysis of the timing of deaths. All that is required is to record the age of death of each member and plot the number of survivors for each age class. Vertical, static, or actuarial life tables differ in that a sample of living organisms (or of ageable remains of dead organisms) is collected over a series of ages¹⁰. The critical assumption of the vertical life table is that the proportion of organisms within each age class decreases as a function of the age-specific death rate.

Van Valen claimed that his data were compiled in cohort-type tables, but Raup³ has argued that Van Valen used vertical life tables for extinct taxa. In fact, Van Valen combined the two types of life table analysis for both living and extinct taxa. The abscissa of his plots for extinct taxa is time of survival, arranged in such a way that all taxa are scaled as if they had originated simultaneously. For living taxa his abscissa scaling procedure is a straightforward reversal of the procedure for extinct taxa, since all living taxa are contemporary but evolved at various times in the past. In both cases the data seem to form a cohort, but they clearly do not. In the case of extinct taxa the scaling procedure is entirely responsible for the appearance of a cohort, but since the actual times of origin of all points are not the same, there can be no cohort analogous to an ecological life table. In reality the data constitute a vertical life table plotted to appear as a cohort, giving a survivorship curve without additional calculations. In the case of living taxa the data form a reversed cohort table, enabling survivorship to be estimated

directly, assuming that such a reversed table is meaningful.

Van Valen's graphical analysis does not contain the usual interpretation of survivorship probabilities. Ordinary survivorship analysis is specific for a particular sample of organisms over a short time interval and a reasonably constant environment. Thus, analysis of survivorship and mortality reveals patterns of life history in relation to some set of environmental (biotic and abiotic) interactions. This is why log-linear survivorship curves of any kind of organisms are rare, since log-linear curves imply that all members have an equal chance of dying regardless of age. Van Valen's plots are fundamentally different. The time scale is in tens of millions of years, but environmental conditions have not been anything near constant over such long time spans¹¹. Furthermore, members of the 'population' (the set of data points) do not necessarily share many common characteristics. They are hardly a homogeneous group reacting to a set of environmental conditions in the same way; they do not necessarily coexist or even share similar environments. Van Valen's survivorship curves aggregate greatly both the time-age scale and environmental conditions.

Given the scaling used by Van Valen, log-linearity is to be expected regardless of whether the probability of extinction of a taxon is or is not independent of age. Four levels of aggregation contribute to the appearance of log-linearity. (1) The environment-taxa relationship is lost by using taxa that have existed in different places at different times and aggregating them into the analogy of a population; environmental variations over time and space are effectively eliminated. (2) The long time scale ensures that nonlinearity could result only from extraordinary irregularities in extinction rates lasting for epochs. Even major waves of extinctions such as occurred at the Permian-Triassic and Cretaceous-Tertiary boundaries are reduced to small irregularities in the curves. (3) Elimination of points from both ends of the curves (representing taxa of relative short existences and those surviving for very long periods) reinforces linearity. Raup⁵ has already made this argument effectively. (4) The use of high order taxa rather than lower level taxa (ideally, species) makes the data points insensitive to variation among taxa and among the environments.

Because of their high level of aggregation, Van Valen's curves effectively eliminate all but the most extraordinary events, that is, they are relatively insensitive to perturbation. It is the aggregated scale itself which is responsible for log-linearity. The inclusion of

stochastic events to explain variation then requires a very broad definition of 'stochastic'.

At least three different questions must be distinguished in discussions about extinction probabilities. One question is whether the probability of survival of a taxon or group of taxa is the same at all geological times. This question must be answered in the negative—the fossil record shows that during periods such as the Permian-Triassic boundary any existing taxon had, on the average, a greater probability of becoming extinct than at other geological time. A second question is whether, at any given geological time, the probability of extinction is the same for all taxa of a given group. The answer to this question is likely to be also in the negative—at any given time, different related taxa may have different probabilities of becoming extinct. The third question is whether at any given geological time, the probability of extinction for any one taxon is independent of its age. Evolutionary theory predicts an affirmative answer to this third question. Natural selection promotes adaptation to the current environments of a species but does not anticipate the future. Confirmation of this prediction must await further studies.

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VAN VALEN REPLIES—Foin, Valentine and Ayala¹ have produced a curious mixture of misinterpretation and conceptual confusion. I was careful² to avoid saying that 'the probability of extinction of a taxon is independent of its age'. This is true only for the mean probability (propensity) for a set of taxa, and I gave reasons³ for believing that individual taxa do often have different probabilities at different ages.

The reasons derive from both evidence and conventional theory, which therefore does not predict constancy as Foin *et al.* claim. Some subtaxa, for example those that are widespread and that contain many species, do have a low probability of extinction, as expected. Together with the observed mean constancy, this requires that the distribution of probabilities of extinction itself be regulated over time², so that the mean probability for older taxa is the same as that for younger ones. Some taxa therefore become more susceptible with age.

The critics seem not to have heard of composite life tables, which are a standard type³⁻⁵. In this type the data are asynchronous and must usually be analysed by the dynamic method. Simpson⁶ first made composite life tables of taxa from the fossil record. Raup⁷ did not, as claimed, make the elementary mistake of believing I treated extinct taxa by vertical analysis, although I disagree with part of what he did say.

One of the few advantages in using the fossil record for biological analysis is its averaging effect⁸. Local spatio-temporal peculiarities are indeed averaged out. This enables general properties to reveal themselves while 'noise' is minimised.

Any realistic combination of taxa with different characteristic probabilities of extinction gives a concave curve (convex in the terminology of analysis). The fact that the curves are rarely concave (and then only for groups with other evidence of heterogeneity, for which the same subgroups are found as for survivorship analysis) implies that the mean probability is roughly constant over long intervals. There are many causes of the various small irregularities in the curves (ref. 2 and L.V.V., unpublished). Low stratigraphic resolution makes the upper left extreme of the curves unreliable, and the low numbers of taxa at the bottom of a log scale make exceptions at the lower right extreme, even when real, of little importance. Appreciable non-linearity is nevertheless easily detectable; I have in fact detected ostensible exceptions in both directions², which do not affect the interpretation of a long term constancy.

Foin *et al.* have a somewhat subtle confusion reminiscent of the denial⁹ of a long term constancy in protein evolutionary rates. In neither case will "capricious" factors¹⁰, even acting over a long time, give the observed similarity in mean rate for different long intervals. They give a quasi-random walk of rates that is unconstrained by any predictable controlling distribution and so diverges wildly. Even an ordinary Markovian random walk usually diverges and so would usually

give a nonlinear curve. The observed degree of similarity can occur only by the existence of feedback control on a single mean value that is stable over long intervals¹¹ in spite of 'random' perturbations, and it is the very existence of this regulated mean which is the most interesting aspect of both kinds of phenomena.

The claim that I didn't use "lower level taxa (ideally, species)" is strange. I used all available and adequate data: 5 graphs were for species, 34 for genera, and 17 for families. The evidence of constancy is similar for each category. Because supraspecific taxa have different numbers of species, their observed linearity is evidence² for the ecological reality of supraspecific taxa.

I gave the first demonstration² that the mean probability of extinction (and that of branching) of subtaxa of a higher taxon varies in geological time. All previous studies had confounded either the absolute number of subtaxa, or the length of geological periods, with this probability. I also gave evidence that there is real heterogeneity in the probability of extinction of contemporaneous subtaxa.

Here is an apparent paradox: individual subtaxa vary both contemporaneously and over time in their susceptibility to extinction, and the mean susceptibility varies over time, yet the mean is constant over long intervals. The Red Queen's Hypothesis^{2,11,12} attempts to resolve the paradox by treating evolution as mostly ecologically rather than genically controlled, and as a zero-sum game of a kind which game theory has ignored. (In response to an earlier discussion¹³ what else is needed for the resolution is simply an independent maintenance of taxonomic diversity, which is an empirical fact whatever its detailed explanation.) As a by-product this ecologically selectionist theory explains the major features of molecular evolution at least as well as the genically neutralist and genically selectionist theories. Some of these congruences have been published¹¹, and I have found that one of the two apparent difficulties¹¹ of the hypothesis does not exist when explicitly tested¹⁴.

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Sodium flux, action potential and temperature dependence

LANDOWNE¹ and Cohen and Landowne² reported that extra Na flux during the action potential in squid axons increased little with decreasing temperature, the overall experimental Q_{10} being about 1/1.4. Hodgkin and Huxley³, however, computed a Q_{10} of 1/2.87 by assuming that temperature affected only the rate constants governing ion permeability changes. This discrepancy between experiment and theory was interpreted^{1,2} as indicating that nerve membranes do not function by changes in ion permeability⁴. We have used Hodgkin-Huxley-type equations³ and incorporated experimental temperature dependencies of the maximum possible Na and K conductances ($\bar{g}_{Na}$ and $\bar{g}_K$) and the nonspecific leak conductance (g_L). Our results seem largely to resolve the discrepancy. A similar resolution of discrepancies between measured and computed K fluxes was proposed by FitzHugh and Cole⁵.

Membrane action potentials were computed with Hodgkin-Huxley equations, modified for *Myxicola*⁶, using a modified fourth-order Runge-Kutta algorithm, and the six-parameter model⁶ (without reference to inactivation parameters determined with conditioning pulses). Q_{10} s of the parameters, from Schauf's results with *Myxicola*⁷, are: 2.64 for Na activation rate constants (α_m and β_m), 2.56 for Na inactivation rate constants (α_h and β_h), 3.02 for K activation rate constants (α_n and β_n), 1.37 for $\bar{g}_{Na}$, 1.50 for $\bar{g}_K$, and 1.34 for g_L . We used these equations because good temperature data were available. We regard them only as an empirical description of the action potential.

Figure 1a and b shows action potentials and the time courses of the Na currents, computed at 5 °C and 15 °C. Table 1 shows temperature effects on time to rise, time to decline, and duration at +50 mV of the action potential. The Q_{10} of the duration at +50 mV (1/2.75) agrees well with the experimental value⁷ of $1/2.47 \pm 0.2$. Also shown are values for net Na movement during the action potential, (pmol cm⁻²) obtained by integrating the Na current curves of Fig. 1a. Taking the Q_{10} of the net Na entry as a measure of the Q_{10} of the extra Na flux, we get 1/1.82 which agrees reasonably well with the experimental value (1/1.4), considering that the preparations differed

and we simulated single membrane action potentials, not repetitive propagated responses. Computations made excluding the temperature dependency of $\bar{g}_{Na}$, $\bar{g}_K$ and g_L gave a Q_{10} of 1/2.44, much as Hodgkin and Huxley³ reported and divergent from the experiments.

We find, therefore, no serious discrepancy between the measured temperature dependency of the extra Na flux during an action potential and that computed

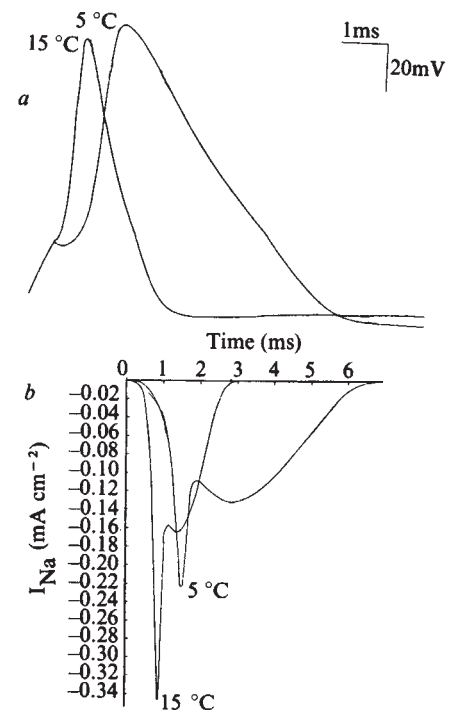


Fig. 1 a, Two computed *Myxicola* action potentials, at the temperatures indicated. Computations have been made according to the six-parameter model of Goldman and Schauf⁶. The experimental temperature dependencies of $\bar{g}_{Na}$, $\bar{g}_K$ and g_L as well as those of the rate constants governing the ion permeability changes have been included. b, Computed time course of the Na currents for the two computed action potentials (a)

from Hodgkin-Huxley kinetics and so major conclusions on the mechanism of ion translocation do not seem warranted. This agrees with reports of a close correspondence between net Na flux determined from voltage clamped currents and

Table 1 Computed temperature effects on the action potential in *Myxicola*

	Time from +25 mV to peak (ms)	Time from peak to 0 mV (ms)	Duration at +50 mV (ms)	Net Na movement (pmol cm ⁻²)
5 °C	0.85	4.38	2.75	5.13
15 °C	0.44	1.73	1.00	2.82
Q_{10}	1/1.93	1/2.54	1/2.75	1/1.82

¹ Foin, T. C., Valentine, J. W., and Ayala, F. J., *Nature*, 257, 514-515 (1975).

² Van Valen, L., *Evol. Theory*, 1, 1-30 (1973).

³ MacDonnell, W. R., *Biometrika*, 9, 366-380 (1913).

⁴ Kurtén, B., *Acta Zool. Fennica*, 76, 1-122 (1953).

⁵ McKinley, K. R., *Am. J. Phys. Anthropol.*, 34, 417-426 (1971).

⁶ Simpson, G. G., *Tempo and Mode in Evolution* (Columbia University Press, New York, 1944).

⁷ Raup, D. M., *Paleobiology*, 1, 82-96 (1975).

⁸ Van Valen, L., *Am. Nat.*, 103, 193-224 (1969).

⁹ Stebbins, G. L., and Lewontin, R. C., *Proc. Sixth Berkeley Symp. Math. Stat. Prob.*, 5, 23-42 (1972).

¹⁰ Lewontin, R. C., *BioScience*, 16, 25-27 (1969).

¹¹ Van Valen, L., *J. molec. Evol.*, 3, 89-101 (1974).

¹² Maynard Smith, J., *Am. Nat.* (in the press).

¹³ *Nature*, 252, 353-354 (1973).

¹⁴ Van Valen, L., *Nature*, 252, 298-300 (1974).

that determined from tracer flux⁸⁻¹⁰, a method which does not use a kinetic model.

This work was supported by a grant from the National Institutes of Health.

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¹ Landowne, D., *Nature*, **242**, 457-459 (1973).

² Cohen, L. B., and Landowne, D., *J. Physiol., Lond.*, **236**, 95-111 (1974).

³ Hodgkin, A. L., and Huxley, A. F., *J. Physiol., Lond.*, **117**, 500-544 (1952).

⁴ Landowne, D., *J. Physiol., Lond.*, **222**, 46p-47P (1972).

⁵ Fitzhugh, R., and Cole, K. S., *Biophys. J.*, **4**, 257-265 (1964).

⁶ Goldman, L., and Schaaf, C. L., *J. gen. Physiol.*, **61**, 361-384 (1973).

⁷ Schaaf, C. L., *J. Physiol., Lond.*, **235**, 197-206 (1973).

⁸ Atwater, I., Bezanilla, F., and Rojas, E., *J. Physiol., Lond.*, **201**, 657-664 (1969).

⁹ Bezanilla, F., Rojas, E., and Taylor, R. E., *J. Physiol., Lond.*, **207**, 151-164 (1970).

¹⁰ Mullins, L. J., Adelman, W. J., and Sjodin, R. A., *Biophys. J.*, **2**, 257-274 (1962).

LANDOWNE AND COHEN REPLY—Goldman *et al.*¹ state that they find no serious discrepancy between the measured temperature dependence of the extra sodium flux during an action potential and that computed from Hodgkin-Huxley kinetics.

We have discussed² this discrepancy, or the lack thereof, in a qualitative fashion and both we² and Fitzhugh and Cole³ concluded that there is still a discrepancy even when the effect of temperature on the maximum conductances was included. Although Hodgkin and Huxley succeeded in predicting the electrical behaviour of the axon membrane based on their measurements of currents resulting from potential steps, they noted that at room temperature there was disagreement between the computed ion fluxes and those found experimentally⁴. We have also computed the fluxes predicted by the Hodgkin-Huxley equations and find there is considerable disagreement with experimental data at many temperatures.

The Hodgkin-Huxley equations were solved every three degrees from 3 to 27 °C and the calculated extra unidirectional tracer fluxes for membrane action potentials are shown as the solid curves in Fig. 1, together with the experimental data. Curve 1 represents the fluxes predicted by the unmodified Hodgkin-Huxley equations with a Q_{10} of 3.0 on the rate constants and 1.0 on the conductances. These calculated fluxes are within 1% of those given in Table 5 of Hodgkin and Huxley's original calculations at 6.3 and 18.5 °C (ref. 4). Curve 2 shows the effect of including a Q_{10} of 1.5 on the conductances and curve 3 was generated by using the Q_{10} s given by Goldman *et al.*¹. By taking the ratio of the net fluxes at 5 and 15 °C from the calculations for curve 3, a Q_{10} of 1.8 is obtained, similar to that of Goldman *et al.*¹. Clearly, none

of the curves fits the experimental data very well although, as suggested previously^{2,3} and by Goldman *et al.*¹, the fit is improved by including the effect of temperature on the maximum conductances. All the computed action potentials look qualitatively similar to experimental action potentials. Thus it seems that the appearance of the computed action potentials is not a good criterion for prediction of the appropriate fluxes.

The experimentally observed Q_{10} s were 1/1.4, 1/0.7 and 1/1.6 for the extra Na influx and 1/1.0, 1/1.3 and 1/1.2 for the extra Na efflux, for a variety of experimental conditions. We think that it is quite possible that these numbers are smaller than 1/1.8.

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¹ Goldman, L., Hahn, R. and Beginisich, T., *Nature*, **257**, 516-517 (1975).

² Cohen, L. B., and Landowne, D., *J. Physiol., Lond.*, **236**, 95-111 (1974).

³ Fitzhugh, R., and Cole, K. S., *Biophys. J.*, **4**, 257-265 (1964).

⁴ Hodgkin, A. L., and Huxley, A. F., *J. Physiol., Lond.*, **117**, 500-544 (1952).

⁵ Moore, J. W., and Ramon, F., *J. theor. Biol.*, **45**, 249-273 (1974).

Equivalence principle

BOTH Bishop and Landsberg¹ and Kilminster in his review² use the argument

Gravitational redshift

→ Retardation of clocks

→ Space-time curvature

where we have identified the "non-Minkowskian metric"¹ and the phrase "the geometry of space-time is modified"² with space-time curvature. We have shown³ this argument (which is due to Schild^{4,5}) to be invalid and gave the rather obvious counterexample of a uniform gravitational field where space-time is flat (since the Riemann curvature tensor vanishes).

Einstein's 1911 paper is not, of course, in the framework of Newtonian physics. The argument

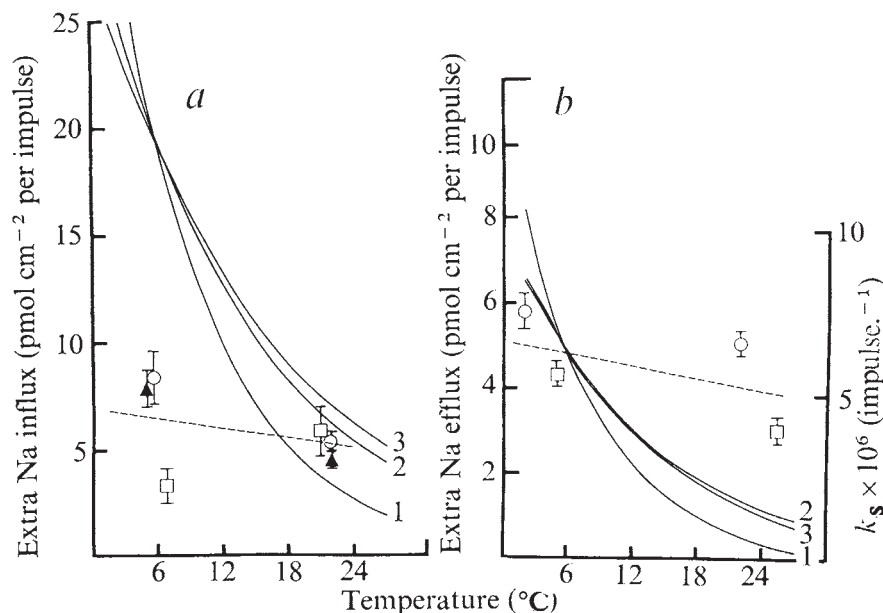
Equivalence principle

→ Retardation of clocks

cannot be applied in Newtonian physics because of the existence of an absolute time. The principle of equivalence itself is only partly contained in Newtonian physics in that "fictitious forces" can be equated with gravitational forces. Since the equivalence principle requires, in addition, that the behaviour of clocks under the influence of a gravitational field and an (locally equivalent) acceleration field be indistinguishable at a point, it may not be fully incorporated into Newtonian physics.

The redshift which arises in the treat-

Fig. 1 Extra unidirectional sodium fluxes associated with nerve impulses in squid giant axons. *a*, Influx: the curves represent predictions made by the equations of Hodgkin and Huxley⁴ solved by a computer program similar to that of Moore and Ramon⁵ with modifications as described in the text. The experimental data (points) are the mean values $\pm$ s.e. from Tables 1 and 2 of Cohen and Landowne². *b*, Efflux: the experimental points represent the mean values $\pm$ s.e. from Table 3 of Cohen and Landowne². The experimental data are plotted against the right hand vertical axis which was obtained from the left hand axis using a diameter of 476 μ m and an internal sodium concentration of 65 mM. The dashed lines were fitted to the experimental points by eye.



ment of accelerated motion requires only the use of the Doppler effect and is merely equivalent to the statement of conservation of energy⁵. Only in special relativity can one equate a Doppler shift with clock retardation which is necessary so that all inertial observers measure the same value for the velocity of light. The principle of equivalence extends this to all freely falling observers.

Furthermore, it can be argued that the full principle of equivalence would be inconceivable before the advent of special relativity: as long as one believed that the velocity of light depends on the motion of the source and/or of the observer, how could one equate an accelerated system with one at rest but in a gravitational field?

We agree with Bishop and Landsberg that relationships between inertial and non-inertial frames are not suitable for discussing the gravitational redshift, and, in fact, this lies at the basis of our treatment in ref. 3.

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¹ Bishop, N. T., and Landsberg, P. T., *Nature*, 252, 459 (1974).

² Kilmister, C. W., *Nature*, 252, 439 (1974).

³ Marsh, G. E., and Nissim-Sabat, C., *Am. J. Phys.*, 43, 266-267 (1975).

⁴ Schild, A., *The Monist*, 47, 20 (1962).

⁵ Misner, C. W., Thorne, K. S., and Wheeler, J. A., *Gravitation*, 187 (Freeman, San Francisco, 1973).

As one of the authors referred to by Bishop and Landsberg¹, I would like to comment before students abandon their text books. Bishop and Landsberg¹ recommend that one should not use Newtonian mechanics with the principle of equivalence, to develop the gravitational redshift. They say that the result "is an absurd conclusion, since Newtonian physics operates with an absolute time". The principle of equivalence also leads to the result that the path of light is curved in a gravitational field. Thus, in another context, it is equally absurd to use the principle of equivalence with special relativity, since light signals are used to synchronise clocks and mark out straight lines in special relativity. Thus, in the presence of gravitational fields, strictly one should only use the general theory. What is done in elementary text books is to add the principle of equivalence on to either Newtonian mechanics or special relativity, whichever is the more convenient. It is an extension in both cases. It serves only as an introduction to the general theory.

A typical approach², considers a light source and detector at the rear and front ends respectively of an accelerating spaceship. The spaceship is considered from

the inertial reference frame in which it is instantaneously at rest, when the light is emitted. Both source and detector have an acceleration a in this frame. The proper length l of the spaceship can be measured using a ruler at this instant. The signal takes a time $t \approx l/c$ to reach the detector, by which time the detector has a speed $v \approx at \approx al/c$ relative to the inertial frame. Provided $v \approx al/c \ll c$, the first order Doppler effect, given by the wave theory of light, is adequate, so that the fractional change in frequency $\Delta v/v \approx v/c \approx al/c^2$.

According to the principle of equivalence, the difference in frequency should be the same, if the spaceship were at rest in a gravitational field of intensity $-a$. Provided $al/c^2 \ll 1$, it is an unnecessary over-elaboration to use the equations of hyperbolic motion, and in a first introductory course the use of Newtonian mechanics and the first order Doppler effect, based on the wave theory of light, is far more convenient. In practical cases $\Delta v/v \sim 2 \times 10^{-15}$ in the experiments of Pound and Rebka and $\sim 2 \times 10^{-6}$ for light from the Sun, so that $al/c^2 \ll 1$, and the simple theory is accurate enough. Near black holes one would have to use the general theory of relativity. This approach, based on the principle of equivalence, should only be treated as a teaching aid, to give a first insight into some of the modifications of both Newtonian mechanics and special relativity required by the general theory of relativity.

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¹ Bishop, N. T., and Landsberg, P. T., *Nature*, 252, 459 (1974).

² Rosser, W. G. V., *Introductory Relativity*, 264 (Butterworth, London, 1967).

³ Pound, R. V., and Rebka, G. A., *Phys. Rev. Lett.*, 4, 337 (1960).

BISHOP AND LANDSBERG REPLY—We shall explain the points raised by referring to the paragraphs in ref. 1 as $a-e$, those in ref. 2 as $a-c$ and those in ref. 3 as $a-g$. We referred^{3b} to a non-Minkowskian metric. This does not imply that we considered the uniform acceleration to produce space-time curvature^{1a}. We had in mind flat (that is, $R_{\beta\gamma\delta} \equiv 0$) non-Minkowskian metrics of the type⁴

$$ds^2 = g_{44}(x)c^2 dt^2 - \frac{c^4}{4\kappa^2 g_{44}} \left(\frac{dg_{44}}{dx} \right)^2 dx^2 - dy^2 - dz^2$$

We thus agree with Marsh and Nissim-Sabat^{1a} that the existence of a gravitational redshift does not imply space-time curvature, but we do not accept that any

such curvature is suggested by us^{3b}. Rosser^{2a} regards curved light ray paths, and therefore accelerated frames, as unacceptable in special relativity. We^{3a} take the opposite view, which is implicitly supported by Marsh and Nissim-Sabat^{1a}.

For the Newtonian case we used a particle picture of light^{3d} in which light particles are emitted with a constant speed relative to the source, and move according to Newton's laws of particle mechanics. The equality of inertial and gravitational mass leads to the equivalence principle for particles, and thus also for Newtonian optics. From our point of view, therefore, the equivalence principle is completely contained in Newtonian physics, as opposed to special relativity where the principle has to be grafted on, since this theory is not about gravity. Our correspondents disagree with this view (and with each other), the view of Marsh and Nissim-Sabat^{1b} being closer to us than that of Rosser^{2a}. For a wave theory of light (advocated in ref. 2c) the velocity of light is constant with respect to an ether and the equivalence principle does not then apply to Newtonian optics, as also remarked by Marsh and Nissim-Sabat^{1a}. The answer to the question raised there is: "By using a particle picture of light".

An important point of our letter was the view reiterated by Marsh and Nissim-Sabat^{1e} but not taken account of by Rosser^{2b}: the replacement of a uniform gravitational field by a uniform acceleration for source and detector is always magically transformed to a Doppler effect argument for an accelerated detector, thus neglecting the motion of the source. This is a non-trivial step since two times are needed at the source if a frequency is to be defined there. Thus the switch from one model to the other has to be justified, and if presented clearly to beginners^{2c} ought to elicit from them a request for a proof. Our work⁴ may be regarded as providing such a proof for special relativity and, in so far as this had never been done in the past, one was faced by a misuse of the equivalence principle. For Newtonian physics we show⁴ that it is this magical switch in the usual argument that leads apparently to the absurdity of clock retardation by way of the Newtonian Doppler effect. As developed by us⁴, one finds a zero Newtonian gravitational redshift but a non-zero Newtonian Doppler effect for an accelerated detector, and the usual equations for special relativity. These results seem to us clear and consistent.

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¹ Marsh, G. E., and Nissim-Sabat, C., *Nature*, 257, 517-518 (1975).

² Rosser, W. G. V., *Nature*, 257, 518 (1975).

³ Bishop, N. T., and Landsberg, P. T., *Nature*, 252, 459 (1974).

⁴ Landsberg, P. T., and Bishop, N. T., *Foundations of Physics* (in the press).

reviews

IN the study of many subjects, including geography, psychology, statistics, eugenics and meteorology, the name of Francis Galton receives mention from time to time. But few, in their training, receive more than a glimpse of the life and work of this somewhat elusive man. Who was he? Where can one gain a comprehensive picture of him? A visit to the library soon leads to the biography by Karl Pearson, completed in 1930, but this work (2,000 pages) is too much for many readers to face. Thus, for many he is still not within reach. But now the position changes, with the timely publication of this book, and at last we have a manageable and readable account; and one which, although only one-sixth of the length of Pearson's work, is nevertheless much more objective.

The first chapter is concerned with the family background and the life of Galton up to the age of 22 (when his father died). Succeeding chapters describe Galton's years as an explorer, in Egypt, Syria and South West Africa, and contain some fascinating accounts of his experiences. One result of these explorations was the production of the famous book entitled *The Art of Travel*, a book which was of great practical value to those contemplating travel in unexplored territory.

After the years of exploration Galton had much to do with the Royal Geographical Society, and with the development of geography as an academic subject; and he played a large part in having the subject introduced at

Francis Galton: psychology of a polymath

Francis Galton: The Life and Work of a Victorian Genius. By D. W. Forrest. Pp. x+340+16 plates. (Elek: London, November 1974.) £5.50.

Oxford University. He also became interested in meteorology, and here he demonstrated his capacity for original scientific research. He studied weather data brought back from Africa by explorers and this led to the discovery of the anticyclone. In 1863 he published a book, *Meteorographica*, containing hundreds of diagrams of climatic conditions in Northern Europe during December, 1861.

By the mid 1860s Galton began to develop an interest in heredity (there is some evidence well documented in this book that the development coincided with a growing realisation that his marriage would probably be infertile). Two chapters carefully trace this interest.

Galton was to a large extent the father of present-day psychology, and several chapters cover in detail his many ideas and experiments in this

field. He was also a pioneer in the development of anthropometry and, at his own expense, set up an anthropometric laboratory as part of the International Health Exhibition held in 1884. His work in this field proceeded with much vigour during the following years and an outstanding development was the technique of personal identification using fingerprints. This work is treated in a chapter devoted to the subject. In 1883, Galton published his last important technical book, *Fingerprints*.

In May 1904 Galton delivered a lecture (at the London School of Economics) to the newly formed Sociological Society, entitled "Eugenics, its Definition, Scope and Aims". This was the birth of the subject of eugenics, and Galton contributed much to the development of this subject. He was by this time, however, into his eighties and his health was on the decline. He died in 1911 at the age of 89.

Professor Forrest's book will be of interest to students drawn from various disciplines. It includes a detailed account of Galton's inventions of various pieces of apparatus, and a bibliography of his published works. It reveals a great deal about the psychology of one who pioneered a good deal of modern psychology (especially with emphasis on the experimental approach), and also a lot about Victorian society in general. The book will be of interest to historians as well as psychologists, biologists and statisticians.

L. S. Goddard

NUCLEAR form-factors measured in elastic electron-scattering experiments decrease with increasing momentum transfer at a rate determined by the nuclear size. If one looks instead at the inelastic region, where the electron loses large amounts of momentum, q , and energy, ν , the cross-section is dominated by quasi-elastic scattering from the individual nucleons in the nucleus, and the q -dependence is a measure of the proton size. In analogous experiments with a hydrogen target, Stanford physicists claimed that in this deep inelastic region they could 'see' the constituents of the proton itself; and the q -dependence indicated that these partons were point particles. Bjorken scaling was observed; apart from kinematic factors the cross-section in this region depended only upon ν/q^2 rather than the independent variables.

Kindergarten parton model

Theory of Lepton-Hadron Processes at High Energies: Partons, Scale Invariance, and Light-Cone Physics. (Oxford Studies in Physics.) By Roy Probir. Pp. x+172. (Clarendon: Oxford; Oxford University Press: London; February 1975.) £6.

Some five years later this "kindergarten parton model" is still very much with us, and Dr Roy's book is a description of these intuitive ideas with their experimental consequences, and the more formal concepts, of scale invariance and

light-cone algebra which have developed from them. The book is likely to be useful to second-year graduate students and 'above'; in the early part it requires knowledge of simple quantum electrodynamic calculations but towards the end the renormalisation group in field theory is called for.

Any introduction to a rapidly changing field can expect to be overtaken by events. Thus, if the author had written the book after the discovery of the ψ particles last November, he would doubtless have stressed the charm quantum number much more. Scaling has influenced the interpretation of deep inelastic proton-proton scattering, but the author has wisely confined himself to lepton scattering where a basic formalism exists and is likely to be of some lasting value.

Colin Wilkin

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Heavy nuclei

Heavy Nuclei, Superheavy Nuclei, and Neutron Stars. (Oxford Studies in Nuclear Physics.) By J. M. Irvine. Pp. 158. (Clarendon: Oxford; Oxford University: London, July 1975.) £8.

THE prospective reader who is attracted to this book by the words "superheavy nuclei" or "neutron stars" is likely to be disappointed. Of its 158 pages of text only one chapter of 8 pages is concerned directly with superheavy nuclei, and a further 9-page chapter is devoted to neutron stars. What it does contain is an excellent review of the current theories of the properties and structure of heavy nuclei.

The book concentrates on the region of heavy nuclei beyond ^{208}Pb and as is pointed out in the introductory chapter this contains only ten naturally occurring elements, although a further 14 have been artificially produced in the laboratory. It is perhaps disappointing that the book contains no hint of the excitement and controversy which have surrounded the discovery of these heaviest elements.

The second chapter contains a brief but thorough review of the observed systematics of heavy nuclei such as binding energies, half lives,

decay modes and fission properties. The third chapter then goes on to give a very detailed discussion of the nuclear models, such as the liquid-drop and individual particle models, which have been used to explain these systematic features. Nuclear correlations are introduced here and used to bridge the gap between these two very simple models. The following chapter deals with the particular topic of collective rotations and the specific modifications which need to be considered to bring theoretical predictions into quantitative agreement with experiment.

Nuclear stability and shell effects are next considered and this leads very naturally, through a discussion of fission and alpha decay, to the prediction of the possible existence of superheavy nuclei and the final chapter on neutron stars. A short list of references to relevant review papers and books is included.

The book is written at an introductory level suitable for research students in nuclear structure physics. It is also likely to be of interest to more experienced workers in this field as providing an extensive and thorough review of current theories of the properties of heavy nuclei with a hint and hope of discoveries yet to come.

C. J. Batty

Come back Aristotle

Confrontation of Cosmological Theories with Observational Data. Edited by M. S. Longair. (International Astronomical Union Symposium No. 63, Cracow, Poland, 1973.) Pp. xi+382. (Reidel: Dordrecht and Boston, Massachusetts, 1974.) Dfl.115; \$38.50.

THIS symposium, held in 1973 to commemorate the 500th anniversary of the birth of Copernicus, provides an excellent picture of cosmology today—of a science caught, as Zeldovich says in his introduction, at a turning point. The programme of Sandage and Tammann to extend the redshift-distance relation for galaxies, the close agreement between the expansion time of the Universe and the age of our Galaxy and other galaxies, and the 2.7 K blackbody spectrum and high degree of isotropy of the microwave background radiation (reviewed here by Blair, Partridge and Boynton) have together convinced all but the most sceptical cosmologists that we live in a hot, big bang Universe.

In paper after paper the most simple, conservative view seems to be supported. Petrosian argues that the cosmological term, introduced and later repudiated by Einstein, is zero. Tammann demonstrates that the velocity flow field of nearby

galaxies is isotropic and linear. Wagoner shows how the cosmic abundances of those elements and isotopes formed primarily in the fireball (deuterium, helium, lithium) favour the simplest type of model, deuterium, in particular, pointing to the existence of the low-density Universe also indicated by the density of matter in galaxies. Steigmann sets severe limits on the amount of anti-matter in the Universe.

In his amusing introduction Zeldovich sets out what seems to be a very open minded philosophy of cosmology. That we cannot simply solve backwards in time the equations describing the evolution of the Universe, but have to take arbitrary chosen variants of the initial state and follow the theory of its evolution to the present and thus to a confrontation with observations. The snag of this procedure being that it is dependent on the prejudices, likes and dislikes of authors and "perhaps even their subconscious Freudian attitude to such things as order, chaos, antimatter". Hence the importance of the confrontation with observation, in which "false theory fades".

But is it really cosmological theories that are being confronted with observations? Apart from a few dissenting, sceptical and dissatisfied voices (for example, those of Arp, Kellermann,

Omnes and Elvius), only one theory is discussed in this book. Kuhn's gestalt switch has occurred and the isotropic big bang has become the paradigm. Within that framework the theoreticians set to work on the details: the problem of galaxy formation (Silk, Doroshkevich, Sunyaev, Zeldovich and Ozernoy), the structure of the initial singularity (Penrose, Novikov, Lifschitz and Khalatnikov), and the exciting work on pair creation by anisotropic expansion (Zeldovich and Misner). This is a situation in which the mathematically brilliant but philosophically conservative Russian school of cosmology flourishes. But is it likely that we shall again have to wait the 2,000 years between Aristotle and Copernicus to see this picture overthrown?

Yet even in the ranks of the believers there are some novel perspectives. Hawking analyses homogeneous, anisotropic perturbations of the standard models and concludes that we must be in a unique model (with zero curvature), arguing that the reason for this must be, in a sense, because we are here. If the Universe was not as it is, galaxies and stars could not have formed and we would not be here to observe it—the 'anthropic principle' developed elegantly in a paper by Brandon Carter.

Come back Aristotle, all is forgiven.

M. Rowan-Robinson

Complex uses of an advanced tool

Quantitative Scanning Electron Microscopy. Edited by D. B. Holt, M. D. Muir, P. R. Grant and I. M. Boswarva. Pp. x+570. (Academic: London and New York, January 1975.) £15.50.

SINCE the early 1960s, the accessibility of the scanning electron microscope (SEM) as a working tool in the hands of scientists has grown tremendously. For example, just one of the several SEM manufacturing companies lists over 500 owners throughout the world, which may imply 10 times as many users. Furthermore, a lot of ingenuity has gone into finding new uses for a tool which yields dramatic topographical pictures suitable for hanging on the wall but can also probe the basic electrical and crystallographic properties of materials in many more subtle ways. This book attempts to review the unique methods by which the properties of inorganic materials may be measured quantitatively using the interaction of a rastered kilovolt electron beam with a solid. It explicitly avoids an introductory approach, but, as parts of it evolved from a lecture course, it is well adapted for an advanced teaching course.

Fifteen authors, mainly from British universities, gave authoritative reviews of each use of the SEM and a strong quadripartite editorial team has successfully imposed unity on the contributions, keeping a sharp eye on the possible future development of the instrument. Several aspects of the production of finer and better controlled electron beams occupy the first three chapters, including an unusual and useful review of the very complicated interaction of electrons with solids and a rather casual run through "the approach to 1 Å" by the renowned Albert Crewe. Six chapters on the quantitative interpretation of contrast borrow from and build upon the art developed for the transmission electron microscope (TEM). Howie's chapter does this by means of a unified survey of diffraction contrast and scattering in the TEM mode (including the scanning and glancing-angle modes) and the backscattering mode.

The emissive mode, for all its importance, is compressed efficiently by Gopinath into only 30 pages, but it would not have been too much of a diversion for the editors to have inserted a chapter on the morphology and physics of crystalline organic materials, thereby capturing a large additional readership.

Finally, the versatility of the SEM is suitably revealed in the section on the use of the X-ray yield of the SEM

sample. This section again gives prominence to creative, forward-looking aspects and, indeed, the final chapter on automatic stereological analysis by Jones indicates that a strong use of the imagination and the help of a computer can tell one what is in the bulk of a mineral conglomerate, working only from the surface X-ray energy analysis. The greatest lack I noticed was of any discussion of the positive side of electron beam damage such as is observed with kilovolt electrons on films of alkali halides. In fact, the editors have stuck rather narrowly to the technologically useful metals, semiconductors and phosphors and the emission therefrom.

This book is not a handbook; I regret somewhat the lack of survey tables and appendices. In general, the editors have not pressed their authors to prepare the information better for their readers. Diagrams are redrawn from the research literature rather than pulled apart, condensed and recombined for clarity. The subediting on diagrams is sometimes loose as to symbols and captions, which may cause the neophyte some crises of confidence. These minor points aside, this is a book which the novice microscopist having grasped the basic principles elsewhere, can use with confidence as a guide to the complex uses of the SEM.

Andrew Holmes-Siedle

Maternal lifeline to foetus

The Placenta and its Maternal Supply Line: Effects of Insufficiency on the Fetus. Edited by P. Gruenwald. Pp. x+366. (MTP: Lancaster, April 1975.) £11.50.

THE declining perinatal mortality in developed countries has highlighted the importance of foetal subnutrition *in utero* as an increasingly common cause of perinatal morbidity and mortality. The present volume is therefore well timed and provides a useful summary of current theories concerning the physiology of foetal growth and possible therapeutic approaches to suboptimal growth patterns *in utero*. Professor Gruenwald has a distinguished record in this area, and was one of the first workers to stress the difference between infants of low birth weight due to premature delivery and those due to intra-uterine growth retardation. The volume consists of chapters written by a variety of contributors—usually

research workers with a particular interest in the subject—and there is therefore no uniform style. Many of the chapters are well illustrated, whereas some have no illustrations at all. The illustrations used by the editor tend to be complicated and difficult to interpret at a glance. The majority of authors present an objective view of their subject but in some cases the author's own viewpoint is emphasised with only cursory reference to opposing schools of thought. The book is well referenced and the bibliography is extensive.

On balance the volume presents a comprehensive review of the current literature on placental development and pathophysiology, as well as a shorter section on foetal growth and pathophysiology, not only in human but in other species. The authors emphasise certain areas in which further research is needed, and particularly the need for new diagnostic and therapeutic possibilities in pregnancy. At £11.50 the volume is recommended as a valuable addition to every medical and biological sciences library as well as to interested investigators.

Y. B. Gordon

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obituary

John Ray Dunning, a pioneer in the development during World War II of the method of isolating uranium 235 used in nuclear weapons and power plants, has died at his home in Florida. He was 73.

Dr Dunning started his graduate career at Columbia in 1929 and was appointed assistant professor in physics in 1935. He toured many European nuclear physics research establishments during the following year and was to become associated with Bohr and Fermi on his return to America. He directed the development of Columbia's first cyclotron, built in 1936, which was to be used in the university's history-making experiments. In determining that uranium 235 is the isotope which readily undergoes fission into

two atoms of nearly equal size and that the reaction releases prodigious amounts of energy, Dr Dunning helped to lay the foundations of the atomic age. He was essentially confirming the results of the enemy in Germany, but his experiments were nevertheless of paramount importance in winning the race to production. He was promoted to full professor in 1946 and in that year served as special representative for the Manhattan District at the first postwar detonation of an atomic bomb on Bikini Atoll. During 1941-46, he was an official investigator of the Office of Scientific Research and Development. From 1942-45 he was director of research in the substitute alloy materials laboratories, which was the official name for the secret gaseous

diffusion project to isolate uranium 235 from 238 for the production of atomic bombs (this process is still the major source of such "fuel"). In 1945 he was appointed director of Columbia's division of war research, and the following year was awarded the Medal of Merit, the highest award made by the US President to civilians. In 1950 he saw the completion of the university's 385 million electron volt synchrocyclotron at Nervis, New York after serving as scientific director of the project.

After 19 years as dean of the faculty of engineering, during which time he had raised over \$50 million for the research effort, he resigned to become Thayer Lindsley Professor in applied science in 1969.

announcements

International meetings

December 1-2, **DNA-repair and late effects**, Vienna (International Atomic Energy Agency, 1010 Vienna, Kartner Ring 9, Austria).

December 1-5, **Nuclear techniques in exploration extraction and processing**, Rio de Janeiro, Brazil (International Atomic Energy Agency, 1010 Vienna, Kartner Ring 9, Austria).

December 2-3, **Grass weeds**, Paris (M. R. Faivre-Dupaigre, 8 Avenue du President Wilson, 75116 Paris, France).

December 3-5, **Cardiology**, Houston (The Office of the Director, The University of Texas Health Science Center at Houston, Division of Continuing Education, PO Box 20367, Houston, Texas 77025).

December 11-12, **Recent advances in chemistry and technology of fats and oils**, Liverpool (Head of Chemistry Department, Liverpool Polytechnic, Byrom Street, Liverpool L3 3AF, UK).

December 16-18, **Structure and function of membranes**, London (Dr J. C. Metcalfe, Department of Pharmacology, University of Cambridge, Medical School, Hills Road, Cambridge CB2 2QD).

Reports and publications

Other Countries

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The Winged Bean: a High-Protein Crop for the Tropics. Pp. vii + 41. (Washington, DC: National Academy of Sciences, 1975.) [158]

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